EFFECT OF VACUUM PACKAGING ON THE SHELF LIFE OF PEARLSPOT (*Etroplus suratensis*) AND BLACK POMFRET (*Parastromateus niger*) DURING CHILL STORAGE

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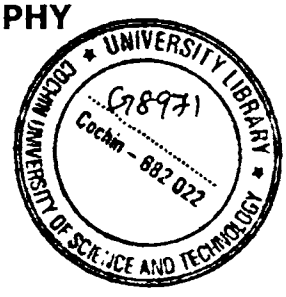
In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY



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CERTIFICATE

This is to certify that this thesis entitled "**EFFECT OF VACUUM PACKAGING ON THE SHELF LIFE OF PEARLSPOT (*Etroplus suratensis*) AND BLACK POMFRET (*Parastromateus niger*) DURING CHILL STORAGE**" embodies the original work conducted by Smt. MANJU. S., under my guidance from 20.08.2001 to 05-01-2004. I further certify that no part of this thesis has previously been formed the basis of award of any degree, diploma, associateship, fellowship or any other similar titles of this or in any other university or institution. She has also passed the Ph. D qualifying examination of the COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY, Cochin held in August, 2003.

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DECLARATION

I, MANJU. S., do hereby declare that the Thesis entitled **"EFFECT OF VACUUM PACKAGING ON THE SHELF LIFE OF PEARLSPOT (*Etroplus suratensis*) AND BLACK POMFRET (*Parastromateus niger*) DURING CHILL STORAGE"** is a genuine record of bonafide research carried out by me under the supervision of Dr. T. K. Srinivasa Gopal, Principal Scientist, Fish Processing Division, Central Institute of Fisheries Technology, Cochin-682 029, and has not previously formed the basis of award of any degree, diploma, associateship, fellowship or any other similar titles of this or any other university or Institution.

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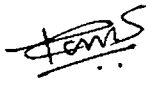

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INTRODUCTION

1.0. INTRODUCTION

Fish are perhaps one of the most vulnerable of the world's resources. For many economically developing nations, fish are the first or second largest export commodity. As a healthy alternative to other animal protein, the seafoods are on the increasing demand all over the world. Fish and fish products are now transported between nations and hence the freshness or quality of these products is becoming more and more important, as it reflects on the price. Current fish utilization trends in India indicate that the major quantity of fish is marked in fresh form. Presently 39.7% of world fish catch is consumed fresh, 8.7% is used for canning, 7.3 % for curing, 20.0 % for freezing and 24.2% is used for other purposes (FAO, 2002).

Fisheries production has an important place in Indian economy. Besides a rich source of protein, it provides income and employment to millions of fishermen and farmers, particularly in the coastal states. Inedible fish in the form of fish meal is also a rich source of animal protein for livestock feeding. The world fish production was around 125 million tons. The major fish producing countries are China, Japan, India, USA, Russian Federation, Indonesia and Chile, and India ranks 3rd in world fish production. At present, India's total annual fish production is about 5.65 million tons (Inland - 2.82 million tons and marine - 2.83 million tons). However, the estimated potential based on the present levels of productivity is about 8.4 million tons (Inland 4.50 and marine 3.90 million tons). The fish exports from India are rising.

The exports go to some 70 countries, with Japan being the single largest market. The export earnings, which were Rs 51170 million in 1999-2000, have increased to Rs 63000 million in 2000-01.

Fish is an extremely perishable food and should be handled with great care to inhibit the growth of micro-organisms. The high ambient temperature of our country favours the rapid growth of micro-organisms. With higher ambient temperature, fish quality deteriorates very rapidly and low temperature storage is the method of preservation recommended to retard microbial spoilage of fish (FAO, 1993). The effect of microbial activity on fresh seafood proteins results in a pronounced off flavour and off odour, which can lead to a short shelf life and economic loss. The availability of whole and processed fresh fish to consumers away from landing and production areas is restricted due to limited storage stability of such products. Retail sales of fresh seafood items are expanding and this necessitates developing alternate preservation methods to extend the shelf life of fresh seafood product. The time factor involved in handling, processing, transporting and holding fresh fishery products has become critical from the standpoint of both quality and profits for marketers of fresh seafoods.

1.1. Vacuum Packaging

Vacuum packaging represents a static form of hypobaric storage which is widely used in food industry due to its effectiveness in reducing oxidative reactions in the product at relatively low costs (Gopal *et al.*, 1999). In

vacuum packaging, the product is contained in a package made of a material having low oxygen permeability and is sealed airtight after evacuating the air. Vacuum packaging brings about a drastic reduction in oxygen content and increases carbon dioxide and acidity. The growth of aerobic spoilage microorganisms is substantially decreased. The vacuum packaging also offers an excellent protection against desiccation and rancidity during storage (Aagaard, 1969).

Vacuum packaging can be defined as a form of MAP since the removal of air is, in itself, a modification of the atmosphere (Wilhelm, 1982; Davies, 1997). In vacuum packages, slow diffusion of atmospheric O_2 through the high oxygen barrier film leaves only residual O_2 (1%). This amount is absorbed through chemical reactions with compounds in the product, through any residual respiratory activity in the product and microbial activity (Adams and Moss, 2000). The residual levels of CO_2 (10% or 20%) in the packages are due to the metabolism of the product tissue and microorganisms (Silliker and Wolfe, 1980; Smith *et al.*, 1990).

It should be stressed that vacuum packaging must be used under strict conditions of hygiene and control, and not as a means to forgo proper sanitation. This could be applied to cook-chill food systems to increase food quality and microbiological assurance. Vacuum packaging in gas impermeable and heat stable materials included no or low risks of post pasteurization contamination, inhibition of growth of aerobic spoilage organisms & inhibition or slowing of deleterious oxidative reactions in the food during storage due to

oxygen barrier properties of the packaging material. There are number of criteria required for the films used for vacuum packaging in large scale production methods, especially those which require in-pack pasteurisation (i.e. pasteurisation of the food after it has been packaged). These requirements include:

- High durability. i.e. ability to withstand considerable mechanical stresses during packaging, handling and transport.
- Retention of flexibility even at low temperatures (-2 to 4°C) to enable satisfactory handling in the packing and refrigeration rooms.
- Ability to withstand heating to at least 150°C without structural damage, leaching of potentially toxic plastics or plasticisers.
- Impermeability to liquids, including oils and fats and macromolecules.
- Impermeability to gases, in particular oxygen, so that oxidative deterioration of the packaged foodstuffs is limited or inhibited.
- Manufactured from non-toxic, food acceptable, odourless materials.
- Must be able to create airtight durable heat seals to close packs.

Many of these criteria, have been met by a range of materials, mostly multilaminated plastics. A wide range of materials are now manufactured by a number of companies throughout the world which are suitable or even specifically designed for use in large scale vacuum packaging cook chill operations. The extreme perishability of fish renders it susceptible to losses at every stage from harvest to consumption. These losses appear to be enormous in India. Incorrect handling techniques, inadequate packaging

materials and poor processing accelerate spoilage and in turn consumers are denied quality fresh fish. The use of ice can significantly increase the shelf life of fish at sea and onshore provided adequate fish to ice ratio is maintained. It is important to use insulated container for storage and transportation to prevent melting of ice.

Vacuum packaging fits into an important area of preservation where shelf life is extended without the loss of those important and exclusive properties, which constitute freshness in the consumer's mind, and therefore move the product into a premium bracket. Recent years have seen vacuum packaging increasingly used for the retail packaging of different kinds of products, including fish (Adams and Moss, 2000). Studies have shown that vacuum packaging in combination with icing improves the shelf life considerably. So, the present work deals with the effect of vacuum packaging on the shelf life of commercially important fishes viz., Pearlspot (*Etroplus suratensis*) and Black Pomfret (*Parastromateus niger*) during chill storage.

Black Pomfret (*Parastromateus niger*) belongs to family Carangidae. It is most abundant on the west coast of India and in Indonesia. In India, the most important fishing grounds are located between Mangalore and Tuticorin. Estimated landings of Black pomfret during the year 2003 were 15678 tonnes (Anon, 2003). Black pomfret in frozen form is exported to various countries like Canada, USA, Srilanka, China, Taiwan, Hongkong, Malaysia, Singapore, Japan, Saudi Arabia, UAE, UK, France, Switzerland, Australia etc. India exported 174976 mt of frozen finfish during the year (2001-02) out of which

black pomfret exported constitute 1480 mt (Anon, 2001). Pearlspot (*Etroplus suratensis*) is an important brackish water fish belonging to the family Cichlidae. It inhabits both freshwater and brackish water and is endemic to the peninsular India and Sri Lanka. It is considered to be a delicacy in the state of Kerala with a good market demand. With the boom of backwater tourism, the demand for pearlspot, the high valued food fish in Kerala, is on the increase. The capture fishery of this fish is a minor one and information on the catches is limited. Total landing of fishes from Inland sector was 40365 tonnes out of which pearlspot contributed almost 4000 tonnes.

Even though vacuum-packed product may not develop rancidity in extended periods of storage, it may develop objectionable odours and flavours due to bacterial activity. *Psychrotrophs* and *Psychrophiles* are the major groups of microbes responsible for spoilage of seafoods under low temperatures. Anaerobic bacterial growth is also associated with spoilage of vacuum packaged seafoods. Preventing growth or destroying aerobic spoilage bacteria and food borne pathogens during storage and handling has enhanced quality and safety of refrigerated foods using food additives and bio-preservatives (Gilliland and Evell, 1983). Several works have been carried out on the use of preservative for extending the shelf life of refrigerated meat products. Preservatives such as sodium acetate and potassium sorbate were found to be effective in preventing microbial growth and improving shelf life under different storage conditions (Kim *et al.*,1995 a;b and Chung & Lee, 1981). Trade status of this kind of product in India is poor. A serious problem in

vacuum-packed seafood is the threat of *Clostridium botulinum* activity. Temperature abuse in the storage of vacuum packed or modified atmosphere packaged fresh fish can result in rapid proliferation of *C. botulinum* during storage.

1.2. Objectives:

- To study the suitability of flexible packing materials for vacuum packaging.
- To find out the effect of vacuum packed samples in comparison to air packed samples.
- To study the effect of chemical treatment on shelf life of vacuum packed fish.
- To study the biochemical, textural, microbiological and sensory parameters during storage.

1.3. Scope:

Fish being a quickly perishable food, its shelf life is relatively short. With the growing demand for convenience, the need for off the shelf, ready-to-cook and ready-to-eat packaged foods are constantly on the rise. Consumers of fish prefer to have them in as fresh a state as possible so that the characteristic flavours which they desire, are still retained. Increasingly, all types of consumers are demanding minimally processed foods that are of high order in overall quality, nutritionally superior and easy to prepare. Advantages of prepackaged unfrozen fish are obvious. The shopper would be

able to handle, select required weight, size, species and price and put into the shopping bag along with other commodities such as bread, vegetables and cereals without risk of contamination. Moreover, the contents would be protected against outside contamination. Bulk transportation of fresh fish in ice results in the leaching of soluble nutrients and flavouring compounds. For augmenting the marketing of fresh fishery products at retail level requires novel methods of packing and storage. One such method of current interest is vacuum packaging. Vacuum packaging, a technologically viable method has been developed as a supplement to ice or mechanical refrigeration to reduce losses and to extend the storage life of fresh seafood products.

For a perishable food like fish it is a promising technique as it gives better shelf life extension compared to icing without the transportation problem or excess freight due to ice. Vacuum packaging can create a significantly anaerobic environment that reduces the growth of aerobic spoilage organisms, which generally are gram-negative bacteria such as *pseudomonas* or aerobic yeasts and moulds. These organisms are responsible for off odours, slime and texture changes, which are signs of spoilage. Vacuum packaging also prevents color deterioration in raw meats caused by oxygen. It can also prevent degradation or oxidative processes in food products. There is great scope to use vacuum packaging technology for tropical fishes to extend shelf life. Vacuum packaging in association with chemical preservatives will further enhance the shelf life of fish and fish products. However, collective works on various quality aspects (biochemical,

microbiological, textural and sensory) of vacuum packaged fish of tropical region, under refrigerated storage are found to be scarce. The present work was carried out to see the effect of vacuum packaging on the shelf life of fresh pearlspot and black pomfret stored in ice and also to study the effect of chemical preservatives in extending the shelf life.

These benefits of vacuum packaging allow an extended shelf life for foods in the distribution chain, providing additional time to reach new geographic markets or longer display at retail. Providing an extended shelf life for ready to cook convenience foods and advertising foods as "Fresh-Never-Frozen" are examples of economic and quality advantages.

REVIEW OF LITERATURE

2.0. REVIEW OF LITERATURE

2.1. Spoilage pattern of fresh fish

Although the flesh of newly caught healthy fish is sterile, the skin, gills and, in fish which have recently been feeding, the intestines may carry considerable bacterial loads. Fish and shellfish are more susceptible to spoilage due to the fact that these have sufficient free amino acids, low content of connective tissue and high moisture (Bramstedt, 1961).

Fish spoil through the combined effects of chemical reaction, continuing activity of endogenous enzymes, and bacterial growth. Among the three, the last is apparently the most important factor in producing the most striking and undesirable alteration in the flavour, odour and appearance of fish (Reay and Shewan, 1949). The rate at which both autolytic and microbial spoilage takes place is directly related to the surrounding temperature (Davis, 1995). Having been hauled from water, much of their carbohydrate reserves may already have been converted to lactic acid in the course of prolonged capture struggle. As a result of accumulation of lactic acid, the pH reduces. Values for the ultimate pH postmortem can vary from about 6.0 to over 7.1 depending on seasonal and other factors. There is also variation between species and the pH may fall to less than 6.0 in a few, including halibut, tuna and mackerel (Buttkus and Tomlinson, 1966). As pH decreases, muscle proteins approach their iso-electric point and denaturation begins.

After death, catabolic activity continues and remaining energy reserves decline. Concomitantly, adenosine triphosphate (ATP) level falls and, at a level, which varies with temperature, enzymes which hold muscles in the relaxed state can no longer function. Within hours, depending upon species, condition and temperature, the muscles begin to contract and the fish stiffens in rigor mortis. By the time rigor mortis is resolved, the continuing activity of endogenous enzymes degrade most of the adenosine nucleotides to inosine monophosphate (IMP) that is at or past its maximum concentration (Kassemsarn *et al.*, 1963; Dingle and Hines, 1971). As the degradation sequence continues inosine and then hypoxanthine (H_X) are produced.

Accumulation of IMP is of particular significance because of the synergistic effects, which it is known to have with many flavourous substances (Kuninaka *et al.*, 1964; Yamaguchi, 1987) as well as an inhibitory influence on bitter substances (Woskow, 1969). The initial sweet, meaty and species characteristic flavours of fresh fish reflect the combination of IMP and free amino acids present in the flesh, as well as some sugars and sugar phosphates (Jones, 1969). Decreasing flavour intensity is largely a consequence of the loss of glucose and of hexose phosphates and IMP (Jones, 1961).

The first stage of spoilage is dominated by endogenous catabolic reactions. Although H_X has a bitter taste, autolytic reactions are associated more with loss of those flavours characteristic of fresh fish; it is a phase that can be considered more as a loss of freshness. Aseptically exercised, sterile muscle

remains little changed after reaching a bland, tasteless stage (Herbert *et al.*, 1971) when most non-microbial carbohydrate and nucleotide degradation would have taken place. Beyond this stage, most odours, flavours and other signs of spoilage appear as a result of the activity of the microbial flora of outer surfaces and intestines. Rates of chemical and autolytic reactions increase with temperature, and bacteria that dominate fish spoilage are close to their optimal growth rates at normal ambient temperature for human activity. It has been found that fish spoils more than twice as fast at 5°C, and 4 times as fast at 10°C.

2.2. Ice storage studies of fish

Storage temperature is the most important environmental parameter influencing the growth rate and type of spoilage microorganisms of highly perishable foods such as fish products. The easiest, cheapest and reasonably efficient method of lowering the temperature of fish is by icing. Ice is an effective and ideal cooling medium. Lowering the temperature of the fish greatly retards the bacterial and enzymatic spoilage (Huntsman, 1931). He suggested that improved quality would result if fish were rapidly pre-cooled to a temperature just above their freezing point as by immersion in circulating chilled seawater.

Chilling cannot prevent the spoilage altogether but in general, the cooler the fish, the greater the reduction in bacterial and enzyme activity (Clucas and Ward, 1996). The bacteria responsible for fish spoilage are psychrophilic, so even if fish is chilled at 0°C under the best conditions of handling bacterial

activity can result in severe losses of quality approaching inedibility after 14 - 16 days (Paine and Paine, 1992). The acceptable storage life of each species is affected by many factors including the method of capture, the location of fishing ground, season, the size of fish etc. (Lima dos Santos, 1981).

Ice storage is relatively short-term method of preservation with storage lives varying between a few days to four weeks. Lima dos Santos (1981) and Howgate (1985) reviewed the literature on ice storage studies of fish and attempted to draw conclusions about the storage lives of fish into different broad categories. These reviews confirmed the existence of wide variations in storage lives between species and even within the same species under different conditions. Disney *et al.* (1971) suggested that many tropical fish have longer shelf life when stored in ice compared to fish from temperate waters.

2.3. Fresh fish preservation

Fish being more susceptible for spoilage compared to land animals, should be preserved and processed properly to reduce post harvest loses and thereby increase its demand. The main objective of the preservation methods is to slow down or even stop the spoilage process until consumption. The efficiency of methods depends on its effect in reducing the spoilage rate and thus increasing the shelf life and improving final quality. The selection of a method of preservation is based on the nature and type of fish, economic viability of the method and technical knowledge. Methods commonly employed are:

2.3.1. Chilling

The most common method for preserving fish in the fresh state is by chilling with ice. Even though bulk transportation of fresh fish in ice permits distribution to inland areas, bulk ice holding has a number of disadvantages (i) ice provides only a limited extension of shelf life; (ii) freight cost of bulk ice transportation is expensive (iii) since the fish are not surface protected by over wrapping or other packaging, cross contamination and other forms of abuse cannot be avoided; (iv) drip from melting ice during transportation can result in unhygienic conditions.

2.3.2. Freezing

This is a common method of preservation/retail distribution of fish. The use of freezing temperature (-18°C) to preserve fish is based on the fact that the activities of food borne microorganisms and enzymes can be slowed down/stopped at subfreezing temperatures. The reason for this is that all metabolic reactions of microorganisms are enzyme catalysed and that the rate of enzyme catalysed reactions is temperature dependent. The shelf life of fish can be extended for 6 - 12 months by freezing. However, during frozen storage deteriorative changes may occur due to protein denaturation (Dyer 1946; Acton *et al.*, 1983; Jiang and Lee, 1985). The major disadvantages of freezing are: (i) high energy costs associated with freezing and storage (ii) excessive drip loss when thawing and (iii) textural changes in the fish muscle.

2.3.3. Drying

The method is based on the fact that microorganisms and enzymes need water in order to be active. In preserving fish by this method, the moisture content of fish is lowered to a point where the activities of spoilage and food poisoning microorganisms are inhibited. Dried, desiccated or low moisture foods are those that generally do not contain more than 25% moisture and have a water activity (a_w) of 0.60 or less.

2.3.4. Salting

Salting is often used in conjunction with drying and smoking. As most bacteria cannot grow in salt concentrations above 6%, salting will reduce bacterial action. If the product is salted, there will be a loss of water and water-soluble nutrients during the salting process and further reduction during drying process.

2.3.5. Smoking

It is a preservative procedure, which combines the processes of salting (brining), heating and drying, and finally the addition of smoke itself. Sodium nitrite ($NaNO_2$), in combination with sodium chloride (NaCl), has been used for decades in smoked fish products as a preservative that imparts colour and enhances flavour. These chemicals are also used to prevent the outgrowth, and toxin production by *C. botulinum* types A & E in the smoking process. However, overall effect of the smoking process on fish protein often results in amino acids being affected by any or all of these steps.

2.3.6. Canning

Canning is a method of preservation of foods in which spoilage is arrested by killing the microorganisms present by application of heat and prevention of subsequent contamination, the product being enclosed in a hermetically sealed container. The containers may be made of metal, glass or any other material that is air tight and heatable. Containers made of tin-free steel, glass, tin, aluminium etc can be used for canning. Unlike other common methods of preservation canning alters the nature of the material significantly forming almost new products because of the various treatments the raw materials are subjected to, and the various additives used in process. Metal containers met with some disadvantages like poor barrier properties, pin holing, and poor seal strength. They also impart an undesirable metallic taste to the product on storage.

2.3.7. Irradiation

Another method of fish preservation, which has not been widely used but has been gaining in popularity, is the use of ionizing radiation. Radiation in suitable doses can kill the microorganisms, insects and parasites, which may be present in food and inhibit enzyme activity. Irradiation is not used commercially to any great extent because of the costs involved and consumer resistance.

2.3.8. High pressure processing

This is a method of hyperbaric storage. High pressure can stop microbial growth and reduce enzymic activity. Refrigerated storage of lean fish at high

pressure extends the shelf life considerably. It is widely accepted that conformational changes of protein takes place at higher pressure, which may be responsible for the shelf life extension. High pressure processing destroys the bacteria without changing the nutritive value of the product. However, because of the technical difficulties in building a commercially feasible high-pressure storage unit, this method of preservation has not become popular.

2.3.9. Modified Atmosphere Packaging (MAP)

In MAP, air is replaced with different gas mixtures to regulate microbial activity and / or retard discoloration of the products. Gases commonly employed are CO_2, O_2 and N_2. The proportion of each component gas is fixed when the mixture is introduced into the package; however no control is exercised during storage. MAP can be used as a supplement to ice or refrigeration to delay spoilage and extend the shelf life of fresh fishery products. However, the disadvantages of this technique include, (i) visible added cost (ii) different gas formulations needed for each product type (iii) Temperature control necessary & (iv) special equipment and training required.

2.3.10. Vacuum Packaging

Vacuum Packaging or hypobaric storage may be defined as the packaging of a product in a high barrier package from which air is removed to prevent growth of aerobic spoilage organisms, shrinkage, oxidation and colour deterioration (Genigeorgis, 1985). Vacuum packaging is often also regarded

as MAP in the sense that elevated levels (10-20%) of carbon dioxide are produced within vacuum packages by microorganisms in the food as they consume residual oxygen (Silliker and Wolfe, 1980) or by respiring product. Mostly, it is this retained CO_2 that is thought to exert some preservative effect, inhibiting gram-negative bacteria, yeasts and moulds (Eklund, 1982). Coyne (1933) was one of the early investigators to apply modified atmospheres to fishery products. In a study with pure cultures of bacteria isolated from fish products, he found that carbon dioxide atmosphere markedly inhibited their growth, while normal growth patterns were observed under air or nitrogen atmospheres. Stansby and Griffiths (1935) found that whole haddock stored under 25% carbon dioxide had a shelf life approximately twice that of products handled by conventional methods. Although the fundamental functions of carbon dioxide have not yet been fully elucidated, it is apparent that when exposed to an atmosphere containing at least 20% carbon dioxide, bacterial activity is inhibited although autolytic changes proceed at the normal pace. In case of cod stored at 2°C, a gain of 2 days of storage time with carbon dioxide was observed. Vacuum packaging is used extensively by the food industry to extend shelf life and keeping quality of fresh meat and fish. The shelf life of vacuum packaged meat and fish depends on a number of interrelated factors, specifically the microbiological quality of product and pH of meat and fish at the time of packaging, permeability of the packaging film used, package integrity and storage temperature.

The introduction of vacuum packaging for the distribution and storage of chilled beef has been hailed as the greatest innovation in meat handling during the last 25 years (Taylor, 1985). Vacuum packaging achieves its preservative effect by maintaining the product in an oxygen deficient environment. In anoxic conditions, potent spoilage bacteria are severely or totally inhibited on low pH meat (<5.8). However, their growth on high pH muscle tissue, or extensive fat cover of inevitably neutral pH, will spoil relatively rapidly in a vacuum pack. Vacuum packing can therefore extend the shelf life of primal cuts composed largely of low (normal) pH muscle tissue such as beef and vension by about fivefold over that achieved in air. For other meats and small cuts, only a two-fold extension of shelf life can be safely anticipated.

The main disadvantage of vacuum packaging of meat, from a commercial viewpoint is that, the depletion of oxygen coupled with the low oxygen permeability of the packaging film, results in a change of meat colour from red to brown. Since consumers associate colour with freshness, vacuum packaged meat is not normally sold at the retail level. However, this is not a problem with fish due to the lower myoglobin content of fish.

A relatively limited amount of research is available on the use of hypobaric storage of aquatic products. Huss (1972) compared the quality of plaice held in ice without vacuum packaging with vacuum packed in polyamide polythene pouches. During the 20 days storage period the plaice packed in evacuated bags had the lowest oxygen content, the lowest bacterial count and the

highest quality score and a six days extension of shelf life was obtained. Hansen (1972) found that Atlantic herring and trout stored directly in ice became rancid in 6 days. Fish stored in evacuated polyamide polythene bags did not become rancid during 20 days of storage, but they did develop an objectionable odour and flavours due to bacterial activity. Pretreatment before vacuum packaging has been studied to improve the shelf life of fresh seafood products.

Shewan and Hobbs (1963), Pelroy and Seman (1969), and Matches (1982) reported longer shelf lives by employing vacuum packing for chilled stored fish. Vacuum packaging has been found to substantially reduce oxidative deterioration in frozen fish and fishery products (Hardy and Hobbs, 1968; Yu *et al.*, 1973; Lindsay, 1977; Billinski *et al.*, 1979; Morris and Dawson, 1979). Frozen fish benefit from vacuum packaging by straightforward exclusion of O_2 and elimination of desiccation. An ice glaze can be effective except that as temperatures fluctuate, glaze is lost unevenly and exposed surfaces can soon lose that protection. Combining glaze with vacuum packaging provides a high standard of protection from desiccation and oxidative deterioration (Josephson *et al.*, 1985).

Effectiveness of oxygen excluding atmosphere (vacuum, carbon dioxide and nitrogen) in inhibiting spoilage bacteria was documented for a variety of meat products such as pork, beef, ham, veal and pastrami (Christopher *et al.*, 1980; Hanna *et al.*, 1981; Kemp *et al.*, 1983; Lee *et al.*, 1983; Laleye *et al.*, 1984). Vacuum packaging of wholesale fresh meat is increasingly being

practiced by meat industry, as it reduces shrink loss, protects meat colour and delays microbial spoilage (Mendonca *et al.*, 1989). Dalgaard *et al.* (1993) reported that vacuum and modified atmosphere packaging have been proved to extend the shelf life of chill stored beef, pork and poultry. Nolan *et al.*, (1989) reported that vacuum packaged precooked turkey and pork were more meaty, less oxidized and less rancid in aroma and flavour than meat packaged with 100% nitrogen and 100% carbon dioxide for both short-term refrigerated storage and long term frozen storage.

Eviscerated Atlantic herring and cod were held at 2-4°C in air at 1 atm. and at 0.018 - 0.026 atm. in a hypobaric chamber. Fish held at low pressure retained a highly acceptable odour and appearance for approximately 3 days longer than those stored in air and retained borderline acceptability for approximately 8 days longer than those held in conventional refrigeration (Haard *et al.*, 1982). Fresh fillets of Atlantic cod (*Gadus morhua*) were stored on ice for 0, 3 and 6 days. Subsequent storage was in plastic trays 10 lb under hypobaric condition (20 mm, 10 mm or 6.2 mm Hg) at 1–2°C. Fish were sampled every 3 days for total aerobic and anaerobic plate counts, Trimethyl amine content, moisture, raw quality assessment and taste panel analysis. Results from laboratory and physical analysis indicated that hypobaric storage at the pressure used in this study did not significantly extend the shelf life of cod fillets (Bligh *et al.*, 1984).

Fresh fillets of lean (cod) and fatty fish (Herring and Mackerel) were stored in a hypobaric chamber at –1.1 to –0.55°C under a pressure of 10mm Hg. The

atmosphere was changed twice hourly and had a relative humidity greater than 95%. The growth of bacteria in the fillet was slowed and qualitative shift in the microflora occurred, compared with fillets held at 0°C on ice. The rate of development of rancidity in the fatty fillets was also decreased, because of these changes, 10–15% extension in keeping times of hypobarically stored fillets was observed at 0°C. In this storage environment, the atmosphere was constantly vented, its pressure was reduced, humidity was saturated and the temperature was controlled. The reduced oxygen tensions slowdown the oxidation of lipid and the activities of some undesirable microorganisms. Chilled lean fish such as cod is spoiled by the action of visceral ferments, endogenous enzymes and the microbial flora (Varga *et al.*, 1980). The storage life of wet fish fillets is limited primarily by the activities of the bacterial flora, especially the proteolytic psychrophillic groups. The rate of microbial activity and lipid oxidation in the fish could be affected by storage in a hypobaric environment. A measurable change in the speed of these processes should influence the keeping qualities of chilled fish flesh (Varga *et al.*, 1980). The spoilage rate of herring fillets in low-pressure storage was lower and the storage life was 9% higher than for fillets stored in ice. The slower growth of bacteria and the slow oxidation of the lipid resulted in a measurable increase in storage life of fillets of lean and fatty fish held in hypobaric environment. The spoilage rate of fillets in the hypobaric chamber was further reduced and the shelf life increased at storage temp of −0.55°C. At this temperature, cod fillets were kept for 17 days and herring fillets for 18

days. A further lowering of the storage temperature in the chamber to -1.1°C resulted in another extension of keeping time to 18 days for cod and 21 days for herring fillets (Varga *et al.*, 1980).

The quality attributes of filleted rainbow trout and Baltic herring in over-wrap packages (polystyrene or wood fibre) and vacuum package stored at 2°C were compared by Randell *et al.* (1977). They found that the shelf life of both trout and herring fillets were shortest in overwrap package than in vacuum package. Vacuum packaging was found to extend slightly the microbiological shelf life of trout fillets, but it did not extend the shelf life of herring fillets as compared to overwrap packaging. Bremner and Statham (1983) revealed that vacuum packaging of raw fish products for chilled storage has not resulted in substantial shelf life extension that can be gained with red meat. The shelf life extension of chill stored and modified atmosphere packed cod was experimented by Cann *et al.* (1983).

Quality stability of mackerel based burgers was investigated during 60 days of frozen storage. Burgers were (i) packed under normal (air) conditions (ii) placed in vacuum packaging (iii) made with added antioxidants and packed. It was concluded from chemical and sensory analysis that vacuum packaging with added antioxidant was effective in preserving the quality of mackerel burgers during frozen storage for 60 days (Lee *et al.*, 1993). Sensory and chemical analyses of vacuum packaged sardine burgers have shown that they could be stored for 90 days without significant loss of quality (Ihm *et al.*, 1992). Protein solubility of cod fillets sealed under vacuum was significantly

higher than those fillets sealed in the presence of air during an 18 months storage study at −25°C (Sirois *et al.*, 1991). Wild salmon and farmed rainbow trout both packed in transparent vacuum skin packaging was followed during storage for 6 months at −17°C. Rancidity developed faster in steaks of wild salmon, as compared to steaks of farmed rainbow trout (Andersen *et al.*, 1990). Vacuum packaging was found to be most effective in retarding oxidative rancidity of frozen minces from Gulf menhaden, *Brevoorita patrons* and Atlantic menhaden, *Brevoorita tyrannus*. Vacuum packaging prolonged shelf life of fresh fish by suppressing growth of psychrotrophic aerobes associated with spoilage (largely *pseudomonas*) and samples become unacceptable organoleptically well before the point at which *C. botulinum* could be a problem at 4°C (toxin production is very slow at temperatures less than 10°C). It is emphasized that the success of vacuum packaging is completely dependent on the initial quality of the fish and adequate temperature control, throughout the storage (Clingman & Hooper, 1986).

Lin *et al.* (1996) reported with reference to butter fish (*Peorilus buri*) mince, that vacuum packaging reduces lipid oxidation and FFA liberation. The reduction also prevented the subsequent lipid-protein interaction as indicated by increased water retention during frozen storage study. Huang *et al.* (1994) reported that vacuum packaged channel catfish had significantly lower psychrotrophic bacterial counts than overwrapped fish in ice storage. They also stated that, vacuum packaging slowed down lipid hydrolysis in cat fish

fillets and it not only provides a better external appearance, but also prevents leakage of fish juice and cross contamination of displayed fishes in retail market. The research by Huang *et al.* (1994) also revealed that quality of iced fresh catfish fillets could be improved through vacuum packaging with low oxygen permeable packaging materials.

Individually frozen fish (*Coregonus clupeaformis*) rapidly developed oxidative off flavours when stored without packaging at –12°C, but both vacuum packaging in barrier films and ice glazing significantly suppressed development of oxidized flavours through 24 weeks of storage. Polyethylene pouches significantly improved oxidative stability compared to those stored unprotected. However, vacuum barrier-film packaging gave significantly better protection than polyethylene at both –12°C and –25°C through 24 and 72 weeks respectively (Josephson *et al.*, 1985). While oxygen depletion is effective in retarding the growth of typical spoilage bacteria, there is a possibility that if the product is temperature abused it may become toxic (Wilhelm, 1982). Studies on vacuum/ carbon dioxide flush packaging of fresh fish have shown that the technique introduces no toxicological hazard from *Clostridium botulinum* toxin provided that the temperature is maintained at 0 -2°C (Bannar, 1979).

Vacuum packaging may reduce counts and extend shelf life (Daley and Deng, 1978). Reduced bacterial growth on vacuum packed fish and a change in the spoilage association compared to that in aerobic pack were observed by several authors (Banks *et al.*, 1980; Jensen *et al.*, 1980 and Shalini *et al.*,

2000). The complete removal of oxygen from a pack of fresh meat ensures longer preservation against microbial deterioration than packaging in oxygen. Using a packaging material of low oxygen permeability can reduce oxidation of meat. As with MAP, vacuum packs are leak proof and odour free and offer merchandising advantage (Paine and Paine, 1992). There is considerable interest in extending the shelf life of chilled, packed fish for supermarket trades (Meekin *et al.,* 1982).

2.4. Chemical preservatives

Quality and safety of refrigerated foods have been enhanced by preventing growth or destroying aerobic spoilage bacteria and food borne pathogens during storage and handling using food additives and bio-preservatives (Gilliland and Evell, 1983; Lindgren and Dobrogorz, 1989; Kim and Hearnsberger, 1994). Although there are many food preservative systems available today, there continues to be a need for new systems for fresh fish. Many of the commercially available preservative mixtures for meats and even for shrimp do not work as effectively with fresh fish. Much work has been done to determine the effect of food preservatives on growth of different microorganisms (Sofos & Busta, 1981; Liewan & Marth, 1985). Wide concentration ranges of organic acid salts such as sodium acetate (0.5 – 10.0% w/w), potassium sorbate (0.1 – 10.0%) and sodium citrate (8.0 – 10.0%) have been used, alone or in combination, to extend the shelf life of fresh meat and seafoods (Ho *et al.*, 1986; Ward *et al.*, 1982; Mendonca *et al.*, 1989; Al-Dagal and Bazarra, 1999).

2.4.1. Sodium acetate

Sodium acetate is an approved (USFDA) flavouring and pH control agent. Brewer *et al.* (1992) reported that beef bologna treated with 3% sodium acetate before vacuum packaging and storage at 4°C for 10 weeks had significantly lower populations of aerobic microorganisms compared to untreated sample. Kim and Hearnsberger (1994) reported that combination of sodium acetate and potassium sorbate with lactic acid culture could provide the inhibition required for extended storage, with respect to the growth of aerobic gram–negative bacteria in refrigerated catfish fillets. Mendonca *et al.*, (1989) reported that surface treatment with 10% potassium sorbate, 10% phosphate with or without 10% sodium acetate and 5% NaCl, prolonged microbial shelf life of vacuum packed refrigerated pork chops to more than 10 weeks. Sodium acetate (1%) and combination of sodium acetate and monopotassium phosphate were found to increase the microbiological shelf life of catfish fillets (Kim *et al.,* 1995 a). Kim *et al.* (1995 b) investigated the use of sodium acetate and bifidobacteria to increase the shelf life of catfish fillets. Zhuang *et al.* (1996) observed that 2% sodium acetate had no significant effect on growth of microbes in shrimp but effective in controlling growth of natural flora on catfish fillets.

2.4.2. Potassium Sorbate

A large number of studies have been carried out to determine the efficacy of Potassium sorbate in extending the shelf life of fresh fish (Pedrosa-Menabrito & Regenstein, 1990; Thakur and Patel, 1994; Ashie *et al.*, 1996). It retards

chemical changes due to bacterial growth, prevents off-odours from oxidation occurring in fish during storage, and inhibits the formation of trimethyl amine and other compounds responsible for fish spoilage. Debevere and Voets (1972) reported that addition of potassium sorbate inhibited the TVN and TMA formation and decreased the number of spoilers in pre-packed cod fillets. Bremner and Statham (1983) achieved suppression of spoilage and significant extension of shelf life by the addition of Potassium sorbate to vacuum packed scallops. Sharp *et al.* (1986) studied the effect of potassium sorbate in extending the shelf life of modified atmospheric packed fresh lake whitefish fillets.

Miller and Brown (1983) reported that dipping in a combination of 1% potassium sorbate and 5 ppm chlorotetracyline, followed by vacuum packaging and storage at 2°C was best for retaining fresh properties and shelf life of rockfish fillets up to 14 days. Al-Dagal and Bazarra (1999) reported extension of microbiological shelf life of whole shrimp by 3 days after treatment with potassium sorbate and bifidobacteria. Lalitha *et al.* (2003) observed that a 15 min dip in ice-cold aqueous solution of 0.5% Potassium sorbate and 0.2% citric acid was very effective in reducing total viable count (TVC), *Faecal streptococci* and *E. coli* of farmed freshwater scampi to acceptable levels. Trench sardines dipped in potassium sorbate (2%) before vacuum packaging had a shelf life of 50 days at 4°C, but the untreated were rejected after 26 days (Chinivasagam and Vidanapathirana, 1985).

Regenstein (1982) applied potassium sorbate as part of ice and found that red hake and salmon were satisfactorily stored up to 28 and 24 days respectively. Fey and Regenstein (1982) reported that a combination of 1% potassium sorbate, ice and modified atmosphere of 60% CO_2, 20% O_2 and 20% N_2 at 1.0°C extended the shelf life at least 28 days for fresh red hake and salmon packaged in gas impermeable bags compared with that of these products packaged with no potassium sorbate. Chung and Lee (1981) found that the application of 1% potassium sorbate extended the lag phase but did not alter the spoilage flora of flounder homogenate stored aerobically. The refrigerated shelf life of fresh trout treated with 2.3% potassium sorbate dip was effectively doubled (from 10 to 20 days) when held in semi permeable (laminated high / low density polyethylene bags) packaging in presence of a CO_2 enriched atmosphere as compared with those packaged without potassium sorbate (Barnett *et al.*, 1987). Tomlinson *et al.* (1965) reported that holding freshly caught cod in half strength refrigerated sea water containing 0.2% sorbate resulted in cod fillets with a significantly longer shelf life than that of cod held in refrigerated sea water without added sorbate.

Doell (1962) reported that sorbate inhibited *Salmonella typhinurium* and *Escherichia coli.* Sorbate has been found to inhibit the growth of *Salmonella, Clostridium botulinum* and *Staphylococcus aureus* in cooked, uncured sausage (Tompkin *et al.*, 1974), *S. aureus* in bacon (Pierson *et al.*, 1979), *Pseudomonas putrefaciens* and *P. flourescens* in trypticase soy broth (Robach, 1979), Vibrio *parahaemolyticus* in crab meat and founder homogenates

(Robach and Hickey, 1978), and *Salmonella*, *S. aureus* and *E. coli* in poultry (Robach, 1980). Smith and Palumbo (1980) reported that potassium sorbate inhibited anaerobic growth of *S. aureus* more than growth under aerobic conditions in agar meat model system and was more inhibitory when lactic acid was added. Shaw *et al.* (1983) reported that addition of potassium sorbate to fresh fish inhibited the growth of spoilage organisms such as *Pseudomonas fluorescens*, *Pseudomonas fragi* and the trimethyl amine producer *Alteromonas putrefaciens.*

Sorbate have been shown to prevent spores of *C. botulinum* germinating and forming toxin in poultry frankfurters and emulsions (Huhtanen and Fienberg, 1980) as well as beef, pork and soy protein frankfurter emulsions and bacon (Sofos *et al.*, 1979). Roberts *et al.* (1982) found that 0.26% (w/v) potassium sorbate significantly decreased *C. botulinum* toxin production in a model cured meat system. The effect of sorbate was greatest at 3.5% sodium chloride, a pH less than 6.0, and low storage temperatures. Ivey *et al.* (1978) reported a prolongation of toxicogenesis from *C. botulinum* in potassium sorbate treated bacon. Dipping fish fillets in 5% potassium sorbate/10% tripolyphosphate solution and storing them at elevated CO_2 atmosphere delayed the growth and toxin production by *C. botulinum* than did the use of elevated CO_2 atmosphere only (Seward, 1982). Lund *et al.* (1987) reported that 0.2% sorbic acid alone in meat products at pH 6.5 would not significantly inhibit *C. botulinum* vegetative cells. Blocher and Busta (1983) observed that potassium sorbate sufficient to give undissociated sorbic acid concentrations

of 250 mg/L in culture media at pH 5.5 to 7.0 retarded the growth of proteolytic strains of *C. botulinum* from spores and vegetative cells.

Sorbic acid and its salts have been extensively studied for its use as antimicrobials in poultry (Elliott *et al.*, 1985; Morrison and Fleet, 1985; Robach and Sofos, 1982; Robach and Ivey, 1978; Park and Marth, 1972). Mendonca *et al.* (1989) reported detrimental effects on the colour of fresh, vacuum packaged pork chops that had been dipped in a solution of 10% potassium sorbate. Myers *et al.* (1983) reported that 5% or 10% potassium sorbate solutions, used as a spray or dip for vacuum packaged pork roasts stored at 5°C for 21 days, resulted in 97-99% reduction in psychrotrophic bacteria compared with untreated controls that were not sprayed or dipped. Greer (1982) indicated that dipping fresh beef in a 10% potassium sorbate solution inhibited growth of psychrotropic bacteria and extended retail shelf life by 2 days.

2.5. Quality changes during ice storage

2.5.1. Biochemical changes

The biochemical changes in fish post-mortem are very complex. Several changes take place in the fish muscle constituents leading to changes in texture and flavour producing odoriferous compounds indicative of spoilage. Chemical changes in ice-stored seafoods are mediated by a combination of bacterial action and endogenous enzymatic activity (Flores and Crawford, 1973). According to Siang and Tsukuda (1989), chemical indices are used to

measure components involved in the breakdown process of fish tissue, after death, by internal enzymes. However, because of the biological variations found in different organisms, chemical indices must be used carefully and in conjunction with information from the other tests. Significant variation in the proximate composition of fish is noticed among different species and also within the species. It depends on many factors like age of fish, size, season, spawning, species etc.

2.5.1.1. Drip Loss.

Carbon dioxide inhibits bacterial growth in fish products, but in case of white fish, it tends to be absorbed by the fish tissues, lowering the pH, decreasing WHC (water holding capacity) of the fish and causing consequent drip. Fey and Regenstein (1982) found increased drip losses for red hake, chinook salmon and to a lesser extent, sockeye salmon stored in 60% CO_2, 21% O_2, 19% N_2 compared to air packaged product. Tiffney and Mills (1982) found that packaging in 100% CO_2 increased the rate and quantity of drip loss. In all instances, the quantity of drip loss of fish stored in high CO_2 concentrations was higher at 0°C than 5°C. Randell *et al.* (1997) observed that Baltic herring fillets in over-wrap packages (polystyrene or wood fibre) had lower drip than vacuum packed herring fillets during storage at 2°C. Özogul *et al.* (2004) observed that an increase in drip loss lowered the sensory quality of vacuum packed sardines stored at 4°C.

2.5.1.2. Total volatile base nitrogen (TVB-N)

TVB-N in fish is mainly composed of ammonia and primary, secondary and tertiary amines (Beatty, 1938). Fraser and Sumar (1998) indicated that bacterial catabolism of aminoacids in fish muscle results in the accumulation of ammonia and other volatile bases. Ammonia and primary amines are bound by formalin, therefore this fraction is called the formalin bound nitrogen (FBN). The trimethyl amine (TMA) represents the fraction, which is not bound by formalin (Beatty, 1938). The TVB-N value is used as an index of quality for deciding the state of freshness of fish (along with TMA). A level of 35-40 mg TVB-N /100g of fish muscle is usually regarded as the limit of acceptability, beyond which the fish can be regarded as spoiled (Lakshmanan, 2000). Farber (1965) has reviewed the use of volatile base nitrogen compounds as an index of spoilage. Some workers have recommended volatile base nitrogen in combination with a taste panel for assessing the quality of frozen fish (Jendrusch, 1967). Generally, there is an increasing trend in TVBN values as the fish gets spoiled. The increasing trend of TVBN values was evident in the case of hake (Quaranta and Curzio, 1983). Skipjack tuna and plaice stored in ice showed rapid increase in TVN during 1-3 days of storage and did not show any significant variation till the 9th day of iced storage (Uchiyama *et al.*, 1966). Lakshmanan *et al.* (1984) assessing the quality of fish and shrimp landed at Cochin Fishing Harbour, during a period of 3 years found that 10.1% of the sample had TVN values more than 30 mg %. High value of TVB-N was reported for fresh water fishes

(Joseph *et al.*, 1988 and Bandhopadhyay *et al.*, 1985). Debevere and Voets (1972) reported that addition of citrate buffer to pre-packed cod fillets inhibited the TVN and TMA formation during storage period of 6 days at 0°C. They also studied the level of TVN formed in cod fillet treated with potassium sorbate and packed in film packs of different thickness. They found that an increased oxygen permeability of the packing film does not cause an increase of TVN during first 6 days in potassium sorbate treated cod fillets. However, an increase of TVN between 6^{th} and 12^{th} day is caused by the production of Formalin Bound Nitrogen compounds. Huss (1972) reported a higher TVB-N development in vacuum packed haddock compared to air packed control. Botta *et al.* (1984) reported that there was a definite increase of TVB-N during ice storage of fresh Atlantic cod particularly after 9-11 days. The mean TVB-N concentration at 15 days of iced storage ranged between 17 and 35 mg / 100 g fish. Cobb and Vanderzant (1975) suggested an upper limit of 30mg N/100g for acceptability of fishes such as cod, haddock, eel and sea pike. TVN-values were found to increase during ice storage studies of whole pearlspot (Varma *et al.*, 1983).

2.5.1.3. Trimethyl amine Nitrogen (TMA-N)

Most species of marine fish and shellfish produce in their digestive process trimethylamine oxide (TMAO), which plays role in osmoregulation. TMAO is a tasteless non-protein nitrogen compound whose content varies with the season, size and age of fish. In frozen fish TMAO is reduced by endogenous enzymes to dimethylamine (DMA) and formaldehyde, whereas in iced fish it is

reduced by bacterial enzymes to trimethylamine (TMA), which is associated with a fishy odour (Regenstein *et al.*, 1982; Castell *et al.*, 1971; Lundstrom and Racicot, 1983; Hebard *et al.*, 1982). The concentration of amines in fish tissues is both time and temperature dependent and is related to the deterioration of fish. The determination of TMA as an indicator of freshness (actually of decay) has been a useful criterion for evaluating the quality of fish. TMA-N between 10-15 mg / 100g muscle is considered as the limit of acceptability for round, whole chilled fish (Connell, 1975). A close relationship exists between the numbers of bacteria in the muscle and the amount of TMA-N that is formed (Laycock and Regier, 1971). It can be assumed that the inhibition of TMAO reducing bacteria increases the keeping quality of fish to a large extent (Debevere and Voets, 1972). He also reported that TMA formation was completely inhibited in presence of 0.4% potassium sorbate in pre-packed cod fillets.

A wider range of TMA levels (from 5 to 26 mg/100 g) has been reported for various species of spoiled fish (Castell *et al.*, 1958; Sengupta *et al.*, 1972). In refrigerated storage, TMA-N formation slows down noticeably (Ishida *et al.*, 1976). This alters the TMA-N threshold level at which fish are considered spoiled. In two fish of identical sensory score quality, the one stored at a lower temperature showed a lower TMA-N value (Anderson & Feller, 1949). Cann *et al.*, (1983) also observed lower amounts of TMA on the last day of storage in cod and herring fillets stored at 0°C than in those stored at 5 and 10°C. Reddy *et al.*, (1995) observed that for tilapia fillets packed under air or

modified atmosphere (75% CO_2: 25% N_2) and stored (8°C and 16° C), TMA content increased with storage and reached a high level on the day of spoilage. Regardless of storage temperatures, TMA levels at the time of spoilage were higher in modified atmosphere packed fillets than in fillets packed under 100% air.

Meekin *et al.* (1982) reported that TMA-N content of vacuum packed untreated sand flathead was found to be above 30 mg/100g after 14 days storage at 4°C. Dalgaard *et al.* (1993) reported that TMA concentration at spoilage was approximately 30 mg/100g in vacuum and modified atmosphere cod fillets. It has been found (Tarr, 1939; Shaw and Shewan, 1968) that characteristic odours of spoilage can occur independently of TMA production, depending on nature of bacterial inoculum. It is also possible that production of TMA could occur independently of spoilage, since TMA accumulates in fish muscle as an odourless salt and is converted to the "Fishy" smelling base only in the later stages of spoilage. The ability of bacteria to reduce TMAO to TMA is used as a taxonomic criterion for the identification of non-fermentative *Shewanella putrefaciens* (Lee *et al.*, 1977). According to Jorgensen and Huss (1989) and Dalgaard *et al.* (1993), a concentration of more than 10^8 cfu/g for *Shewanella putrefaciens* is required to produce TMA at a concentration of 30 mg/100g and have perceptible spoilage. Laycock and Regier (1971) observed that *pseudomonas*, the only organism responsible for spoilage of fish at low temperatures have also been implicated in TMA production. Production of TMA-N and pronounced increment in Non Protein Nitrogen (NPN) in the

muscle during cold storage could be used as an indicator of bacterial activity (Gokodlu *et al.*, 1998; Ryder *et al.*, 1984). TMA-N is considered a valuable tool in the evaluation of quality of fish stored in ice because of its rapid accumulation in muscle under refrigerated conditions (Gokodlu *et al.*, 1998; Kryzmien and Elias, 1990).

2.5.1.4. Thiobarbituric acid value (TBA)

A major cause of muscle food deterioration is oxidative rancidity. This oxidative deterioration of muscle lipids involves oxidation of the unsaturated fatty acids, particularly the polyunsaturated fatty acids (Allen and Foegeding, 1981). The polyunsaturated fatty acids, which have three or more double bonds and are associated with the phospholipids are critical to the development of off-flavour in muscle (Allen and Foegeding, 1981; Reineccius, 1979).

The peroxides formed may break down to carbonyls, form polymers, or react with protein, vitamins, pigments etc. (Gray, 1978 and Karel, 1973). Lipid oxidation frequently contributes to flavour changes that occur during the storage of food and is one of the major degradative processes responsible for losses in quality of high-fat foods. The most widely used test for measuring extent of oxidative deterioration of lipids in muscle foods is the 2-thiobarbituric acid test or TBA test, which expresses lipid oxidation in mg of malonaldehyde/Kg of the sample (Sinnhuber & Yu, 1958). Malonaldehyde was shown to be a secondary oxidation product of polyunsaturated fatty acids

containing three or more double bonds (Dahle *et al.*, 1962; Pryor *et al.*, 1976). The discoloration of fish muscle during storage decreases sensory quality (Pokorny *et al.*, 1974) and is more pronounced at higher temperatures. TBA index is the most used indicator for advanced lipid oxidation (Nishimoto *et al.*, 1985). Ryder *et al.* (1984) reported that for mackerel (*Trachurus novaezelandie*) during ice storage the increase in TBA number from 2nd day to 7th day was associated with the detection of oily odours and flavours for cooked fish, and the development of rancid and oily odours of the gills. Nishimoto *et al.* (1985) postulated TBA number of 3.0 and from 4 to 27 for good and low quality fish respectively. Highly significant correlations have been obtained between TBA numbers and taste panel results in various oxidised foods (Tarladgis *et al.*, 1960). Vacuum packaging has been found to substantially reduce oxidative deterioration in frozen fish and fishery products (Yu *et al.*, 1973; Lindsay, 1977).

It can be said that if the TBA value is above 1-2 mg malonaldehyde/Kg of sample, then the fish will, in all probability, smell and taste rancid (Lakshmanan, 2000).

2.5.1.5. K - value

Many methods like TMA-N, TVN etc are used for the objective assessment of fish muscle quality during storage. Martin *et al.* (1978) reviewed that changes due to autolysis and bacterial activity during chill storage were better revealed by the presence of adenosine 5'-triphosphate (ATP) catabolites

rather than by the presence of TMA. ATP of fish muscle breaks down, either during the death struggle or subsequently. Various measurements of adenine nucleotides and their degradation products in fresh fish are used as chemical indices of freshness because the rates of change of these compounds in many species parallel rates of quality deterioration under usual handling of refrigerated fish (Jones *et al.*, 1964 and Dyer *et al.*, 1966). Adenine nucleotides and their degradation products contribute directly to sensory qualities of fish (Jones and Murray, 1964; Spinelli, 1965; Martin *et al.*, 1978; Fletcher and Statham, 1988). The pattern of nucleotide breakdown in most fish species is known to proceed as follows:

ATP→ ADP → AMP→ IMP → Inosine (H_xR) → Hypoxanthine (H_x) → Xanthine→ Uric acid.

The conversion of ATP to inosine 5'-monophosphate (IMP) is very fast and is usually complete within a day (Jones, 1965 and Hiltz *et al.*, 1972). Subsequent accumulation of H_xR or H_x is related to both autolytic and/or microbial action (Martin *et al.*, 1978). Saito *et al.* (1959) were the first to estimate the freshness of muscles from the ratios of the sum of the inosine and Hx to the sum of all other ATP breakdown products. The ratio expressed as a percentage, is called the K value, thus.

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

The use of nucleotide degradation or K-value as an index of freshness have been assessed by Ehira and Uchiyama (1974) in about 100 fish species during iced storage. Comparing nucleotide degradation and sensory analysis in 4 tropical fish species during iced storage, Bremner *et al.* (1988) observed that shelf life and overall acceptability were more related to IMP degradation and K-value rather than bacterial spoilage. In an earlier study Kiesvaara *et al.* (1990) could establish that K-value served as a freshness indicator for several Finnish freshwater species. Ryder *et al.* (1993) and Lakshmanan *et al.* (1993) also found that K-value is a good objective index for the freshness of hoki and rainbow trout, respectively during iced storage. The changes in freshness and flavour components of milkfish (*Chanos chanos*) at various storage temperatures (in ice, 5, 15, 20 & 35°C) indicated that K-value was a reliable indicator for milkfish. Mathew *et al.* (1999) observed an increase in K-value of Atlantic mackerel during ice storage over ten days. Price *et al.* (1991) observed that K-value showed a significant positive correlation with storage time in dressed, bled or round albacore on ice, in chilled seawater and ice slurry at 1.1°C. Greene *et al.* (1990) found a high K-value (80%) in flathead sole after one day in ice. Hattula and Kiesvaara (1992) also observed that the ratio of increase of K-value was very fast in pike, flounder and white bream, where it reached 50% before the third day compared to the K-value of trout, bream and vimba which were still below 50%.

2.5.1.6. pH

In general, dissolution of CO_2 in the fish muscle resulting in the formation of carbonic acid causes a slight drop in pH. Both the buffering capacity of the fish proteins and the composition of the spoilage flora determine the magnitude of pH change (Cutting, 1953). The extent to which pH decrease is proportional to the concentration of CO_2 in the atmosphere (Lannelongue *et al.*, 1982; Tiffney and Mills, 1982). Studies have shown that CO_2 is absorbed rapidly and pH drops over the first two days of storage. The results of pH analysis during storage studies of vacuum or nitrogen packed pastrami, have shown that a significant drop in pH was observed at 3°C. The decrease in pH was usually attributable to the metabolic activity of the lactics, whereas an increase in pH was due to the activity of *pseudomonas spp.* (Jaye *et al.*, 1962). In cod fillets packed under air and under a modified atmosphere of 50% CO_2 and 50% O_2 and stored at 0-2°C, it was found that pH decreased initially due to the production of lactic acid and at the time of spoilage it approached neutral (Gopal *et al.*, 1986).

Parkin *et al.* (1981) noted a drop in pH of rock fish from 6.7 to 6.3 which was maintained throughout the storage period. However, Fey and Regenstein (1982) found that after an initial decrease, fish pH increased and after 27 days storage in a CO_2 enriched environment had reached a level similar to its initial pH. Reddy *et al.* (1994) observed an increase in surface pH from 6.2 to 6.6 after 9 days of chill storage for tilapia fillets packed under air, which indicated bacterial growth and possible spoilage. The increased surface pH in

spoiled fillets may be partly attributed to the production of alkaline compounds such as ammonia by spoilage bacteria (Cann *et al.*, 1983; Scott *et al.*, 1986; Stammen *et al.*, 1990). However, surface pH of fillets held under 75% CO_2: 25% N_2 decreased by 0.22 after 30 days of storage. This decrease may be attributed to the dissolution of CO_2 into the aqueous environment of the fish flesh (Parkin *et al.*, 1981; Statham *et al.*, 1985). Dalgaard *et al.* (1993) reported that during the storage of vacuum and modified atmosphere packed cod fillets the pH increased slightly. Meekin *et al.* (1982) reported decline in pH of vacuum packed sand flat head fillets stored at 4°C after 6 days of storage. Sigurdson (1947) records pH values not exceeding 6.9 for spoiled herrings, which suggests that reduction of acidity proceeds more slowly during the spoilage of fatty fish, probably owing to hydrolysis of fat. Strochecker *et al.* (1937) have noted that the rise in pH during spoilage is greater in fish than in meat.

2.5.2. Microbiological Changes

Microorganisms play an important role in the spoilage of fish (Cann, 1977). Skin surfaces of fish from cold and temperate regions generally have total viable counts of 10^3 to 10^5 cm^{-1}, and aerobic gram-negative *pyschrophiles* and *psychrotrophs* predominate. Two main generic groups, *Pseudomonas/ Alteromonas / Shewanella* and *Moraxella / Acinetobacter*, comprise 60% to over 80% of the flora (Hobbs, 1991). Similar numbers of more mesophilic, gram-positive organisms are found on fish from warm waters (Shewan, 1977; Matches, 1982). Many such fish remain edible for longer than similar cold-

water species, though the pattern of differing storage lives is very complex (Lima dos Santos, 1981). Amongst the various reasons for different storage lives, Shewan (1977) has suggested that one likely cause may be the absence from the initial flora of tropical fish, of many of the "active spoilers" found on fish from cooler waters.

During storage of fish in ice even under the best conditions where the temperature is maintained at 0°C, number of bacteria increased after a lag phase of 1 or 2 days, reaching maximum values of about 10^7 to 10^8/g of muscle after 9 to 12 days (Shewan, 1965). Shewan (1965) and Huss (1972) observed a steady increase in the microbial count during ice storage of haddock. *Pseudomonas* and *achromobacter spp.* were known to be predominant. Spreekens (1974) found that psychrophiles dominated the microflora of whole cod caught by a research vessel and stored in ice. Laham and Levin (1984) observed that 8.8% of the microflora of haddock fillets stored at refrigeration were largely psychrophilic pleomorphic cells. Sulphide producing bacteria have been used as indicators of spoilage (Jorgensen *et al.*, 1988; Capell *et al.*, 1997). Sulphide producers often constitute a major proportion of the microbial flora of spoiling fish, the predominant sulphide-producing bacteria being *Shewanella putrefaciens* (Gram *et al.*, 1987; Gram, 1992). Gram and Huss (1996) reported that *Shewanella putrefaciens* and *Pseudomonas* are the specific spoilage bacteria of iced fish regardless of the origin of the fish. Shewan (1977) reported that *Pseudomonas* species as the

main spoilage bacteria in fish stored in ice mainly because of their short generation time.

Some earlier workers observed that tropical fish had a much longer shelf life in ice than cold water fish (Disney *et al.*, 1971 and Shewan, 1977). This is because the naturally occurring bacterial flora of tropical fish contain only a low proportion of psychrophilic bacteria and the large drop in temperature during icing, had a more pronounced effect on mesotrophic bacteria (Disney *et al.*, 1974; Poulter *et al.*, 1985). But a considerable proportion of the bacterial strains from tropical fish easily adapted to growth at lower temperature. The presence of a large number of bacteria capable of adapting to growth at lower temperature and their biochemically more active nature, contribute to the speedier spoilage of tropical fish, during iced storage. The changes in bacterial flora of marine species such as cod during storage in ice indicate that after a lag period of 2-3 days, there is a logarithmic increase in numbers and by 10^{th} day there is an optimal count of $10^8/cm^2$ of skin or /g muscle. Qualitatively there is little change over the first days, but after this time the *pseudomonas* groups, gradually take over and by 12^{th} day constitute 90 % of the total flora (Shewan, 1971). Surendran and Gopakumar (1991) also observed a steady increase in *pseudomonas*, which approximately constituted 50 to 70% of the total flora in 2 days of ice storage of *Labeo rohita, Labeo calbasu* and *Cirrhinus mrigala*. James (1976) observed that *pseudomonas* could produce proteolytic enzymes in meat at 2°C after 14 days and after 2 days at 25°C. Studies carried out by many workers show that

enzymes produced by microbes interfere with many biochemical changes of fish stored in ice (Chen *et al.*, 1981; Middlebrooks *et al.*, 1988). The odours and flavours of spoiling fish are caused by metabolic waste products of some, not all of the successful organisms as they utilize water soluble constituents of tissues. It is only in the later stages, when spoilage is quite advanced, that tissue proteins are broken down by bacterial proteinases, replenishing the pool of small peptides and free amino acids. Microflora present in the fish stored at 2°C can decrease or increase the pH of muscle tissue depending on the type of microflora (Chen *et al.*, 1981) and pH is also known to influence the protein characteristics.

2.5.3. Microbiological Changes in vacuum packed fish

Carbon dioxide, which has a powerful inhibitory effect on bacterial and mould growth when present in concentrations above about 20%, is the most important gas. The manner in which it inhibits growth is not fully understood, but King and Nagel (1975) showed that some anaerobic and facultative species are also inhibited, indicating that the effect is due to more than just the exclusion of oxygen. Growth of common aerobic spoilage organisms, such as *Pseudomonas, Flavobacterium, Micrococcus* and *Moraxella* are inhibited by CO_2 in modified atmosphere packaged fresh fishery products during refrigerated storage. These psychrotropic bacteria produce chemical spoilage indicators such as trimethyl amine, total volatile nitrogen, hypoxanthine and ammonia (Banks *et al.*, 1980; Brown *et al.*, 1980; Wilhelm, 1982; Oberlender *et al.*, 1983; Cann *et al.*, 1983; Molin *et al.*, 1983; Wang

and Ogrydziak, 1986; Pedrosa-Menabrito and Regenstein; 1988, 1990). Inhibition of these common spoilage psychrotrophic bacteria increases the shelf life by permitting a different type of spoilage flora (e.g. the slower growing gram positive bacteria including *lactobacillus*) to grow. Growth of *lactobacillus sp.* and other gram-positive bacteria under these conditions may result in the souring of fresh fish during storage (Wilhelm, 1982). The inhibitory effect of CO_2 accumulating within a vacuum pack on the growth of *pseudomonas* and other aerobic spoilage bacteria is likely to be reduced when packs are kept at 4°C or above because the organisms are more sensitive to CO_2 at temperatures close to 0°C (Haines, 1933). It was shown by Coyne (1933) and Haines (1933) that 10 - 20% CO_2 delayed the growth of *pseudomonas* and certain other spoilage organisms if the temperature remained below 4°C, ideally close to 0°C. In vacuum-packed iced stored fish from temperate marine waters an increased development of Trimethyl amine is seen while the shelf life is unaffected compared to aerobically stored fish. The number of *Pseudomonas* is reduced, but *Shewanella putrefaciens*, which is capable of anaerobic respiration using TMAO, grows to levels of 10^6-10^8 cfu/g (Gram *et al.*, 1987; Jorgensen *et al.*, 1988; Dalgaaard *et al.*, 1993). In case of cod stored in CO_2 at 20°C, regardless of the packaging method (vacuum or carbon dioxide) TMAO reducing bacteria, including *pseudomonas* and hydrogen sulphide producing organisms dominated in the spoiled fish.

Spoilage of most muscle foods is thought to occur when the aerobic plate count reaches 10^7 cfu/g or more (Ayers, 1960). The qualitative changes

occurring in microflora on aerobic, vacuum packed and CO_2 packed smoked blue cod during storage at 3°C were investigated by Penny *et al.* (1994). Kim and Hearnsberger (1994) reported that growth of undesirable spoilage bacteria in refrigerated fish causes deterioration of keeping quality and reduces consumer acceptance. The H_2S producing *Shewanella putrefaceins* has been identified as the main spoilage bacteria of whole cod stored in ice and in chilled vacuum packed fillets (Gram *et al.*, 1987). Dalgaard *et al.* (1993) reported that large gram-negative microorganisms with psychrophilic characteristics were dominating the microflora of spoiled fillets stored in CO_2 containing atmospheres.

Shewan and Hobbs (1963) found that in fillets of whole gutted haddock, the counts at 0°C and 20°C, became increasingly larger in unpacked fish during storage either at 0°C or in ice, than in vacuum packs. At 12 days of storage, the total aerobic bacterial count and proteolytic count were significantly higher in samples of cod fillets stored on ice, compared to those held at low pressure (Varga *et al.*, 1980). Fillets stored in evacuated containers have been reported to be spoiled by *Pseudomonas* (Pelroy and Eklund, 1976; Licciardello *et al.*, 1966). Bell *et al.* (1995) investigated the growth of psychrotrophic pathogens such as *Aeromonas hydrophilia, Listeria monocytogenes* and *Yersenia enterocolitica* on smoked blue cod packed under vacuum or CO_2. In all vacuum packs, all three psychrotrophic pathogens were able to grow during storage at 3°C. *Lactobacillus* is a predominant spoilage microbial group in vacuum packed fresh or processed meat (Newton

and Gill, 1978). Bremner and Statham (1983) reported that *Lactobacillus* was not present after 13 days of storage of vacuum-packed scallops. Mendonca *et al.* (1989) reported that potassium sorbate is effective in delaying the growth of *Lactobacilli.*

Pierson *et al.* (1979) reported that sorbate delayed the growth of *Staphylococcus aureus* in vacuum packs unlike in non-vacuum packaged hams. Kemp *et al.* (1983) reported that staphylococcal counts of non-vacuum packaged sorbate treated groups were higher than the control group of dry cured intact and boneless hams. Kemp *et al.* (1989) found that at 2°C the staphylococcal counts remained near or below the initial counts throughout the storage of vacuum packaged, dry cured ham slices.

2.5.4. *Clostridium botulinum*

Clostridium botulinum is a species of anaerobic, spore forming, rod shaped bacteria, producing a protein with a characteristic neurotoxicity. The severe food poisoning botulism, resulting from consumption of botulinum toxin have been reported to pose a hazard in vacuum packed fish. There is a large body of evidence, which indicates that fresh fish may be contaminated with *Clostridium botulinum* organisms either as a result of the organisms being present in the microbiota of the fish ecosystem or as a result of post-catching contamination during processing (Bott *et al.*, 1966; Foster *et al.*, 1965; Fantasia and Duran, 1969; Craig *et al.*, 1968; Eklund and Poysky, 1967; Insalta *et al.*, 1967). *C. botulinum* type E has been called fish botulism

organism and has been shown to occur in marine and lake sediments and in fish intestine.

Measures to prevent botulism include reduction of the microbial contamination level, acidification, reduction of moisture level, and whenever possible, destruction of all botulinal spores in the food. Heat processing is the most common method of destruction. Properly processed canned foods will not contain viable *C. botulinum.* A food may contain viable *C. botulinum* and still not be capable of causing botulism. If the organisms don't grow, no toxin is produced. Although many foods satisfy the nutritional requirements for the growth of *C. botulinum,* not all of them provide the necessary anaerobic conditions. Both nutritional and anaerobic requirements are supplied by many canned foods and by various meat and fish products. Growth in suitable foods can be prevented if the product, naturally or by design, is acidic (of low pH), has low water activity, a high concentration of sodium chloride, an inhibitory concentration of sodium nitrite or other preservative, or two or more of these conditions in combination. Refrigeration will not prevent growth and toxin formation by nonproteolytic strains unless the temperature is precisely controlled and kept below 3.3°C. Foods processed to prevent spoilage but not usually refrigerated are the most common vehicles of botulism.

Temperature abuse of commercial vacuum packaged or modified atmospheric packaged fresh fish fillets can result in rapid growth of *C. botulinum* type E spores during storage (1–4°C). These organisms are non-proteolytic and can grow and produce toxin at very low temperatures (3.3°C). A *Clostridium*

botulinum contaminated product may be toxic and still remain organoleptically acceptable without noticeable signs of quality reduction or evidence of spoilage (Lilly and Kautter, 1990). Cann *et al.* (1965) and Post *et al.* (1985) reported the growth and toxin production of *C. botulinum* type E in certain vacuum packed fish. Although the incidence of botulinum from consumption of refrigerated food is exceedingly low, there have been several reported outbreaks associated with the consumption of fish products mainly involving type E toxin (Huss, 1981). Eklund *et al.* (1988) investigated the effectiveness of pasteurization on hot smoked salmon spiked with *C. botulinum* spores B and E. Reddy *et al.* (1997a) investigated the shelf life and toxin development by *C. botulinum* during storage of modified atmosphere packaged aqua cultured salmon fillets.

2.6. Texture analysis

Texture can be defined as "that group of physical characteristics that arise from the structural elements of the food, are sensed primarily by the feeling of touch, are related to the deformation, disintegration and flow of the food under a force, and are measured objectively by functions of mass, time and length (Broune and Szczesniak, 1993). Thus texture can be regarded as a manifestation of the rheological properties of a food. It is an important attribute in that it affects processing and handling, influences food habits, and affects shelf life and consumer acceptance of foods.

Texture of fish is considered to be an important quality attribute for fish products palatability (Howgate, 1977; Cardello *et al.*, 1982). Fish texture differs widely from that of meat because it contains less connective tissue and the cross links formed between collagen molecules are weaker, resulting in a more tender structure (Ashie *et al.*, 1996). Textural changes in fresh or processed fish have been linked to numerous phenomena, such as degradation of myofibrillar proteins (Yamashita *et al.*, 1996) or collagen (Sato *et al.*, 1997), formation of protein aggregates (Gill *et al.*, 1979) or ultrastructural changes (Kanoh *et al.*, 1988; Ando *et al.*, 1991).

There is a long history of efforts to measure texture by instrumental methods, going back at least as far as Lipowitz (1861). Tests that attempt to imitate with instruments the conditions to which the food is subjected in the mouth or on the plate are called imitative tests. It is in this area that texture profile analysis (TPA) falls. A more comprehensive description of the texture of fish is obtained using the Texture profile method (Johnson *et al.*, 1981; Bourne, 1978; Breene, 1975 and Friedman *et al.*, 1963). The first attempt to imitate mastication by instrumental means was the MIT denture tenderometer (Proctor *et al.*, 1955). But, the difficulty with this instrument was that little information other than the peak force could be obtained from the analysis of the resulting force-time curve (Proctor *et al.*, 1956 a; b). The major breakthrough in TPA came with the development of General Foods (G.F.) Texturometer (Friedman *et al.*, 1963; Szczesniak *et al.*, 1963). Analysis of the force-time curve led to the extraction of seven textural parameters -

fracturability, hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness. Each of these parameters identified by General Foods group gave excellent correlations with sensory ratings (Szczesniak *et al.*, 1963).

Bourne (1968; 1974) adapted the Instron Universal Testing Machine to perform a modified texture profile test in a manner analogous to that of G.F. texturometer. This instrument is somewhat flexible, and the supporting platform bends a little as the load is applied; whereas the Instron is rigid, and bending of the instrument is so light that it can be ignored. The G. F., texturometer more closely imitates the action of the human jaw, which has been shown to approximately follow a sine-waved speed pattern (Shama and Sherman, 1973). Szczesniak and Hall (1975) gave examples of the use of TPA on a number of foods and tabulated conditions for use of the G. F. texturometer on 23 different foods. Tanaka (1975) reviewed the use and application of the G. F. texturometer to Japanese foods. A softening of the texture was also observed in several fish species stored at 4°C for up to 14 days, in a study by Hatae *et al.* (1985) using a G.F. texturometer.

Heat treated products are much softer than the fresh product (Huang & Bourne, 1983). Voisey and Larmond (1971) compared various physical techniques for measuring texture of canned beans with sensory evaluation and showed higher correlation. Market research indicates that 75% of buyers (smoke houses, supermarkets, etc.) of Norwegian salmon rate texture as one of the most important quality parameters (Koteng, 1992). Many fish species

do not have a strong flavour and therefore, texture becomes very important for consumer acceptability.

The TPA technique has been a rewarding avenue of research in terms of giving a better and more complete understanding of the textural properties of foods. One great contribution has been its ability to prove conclusively that texture is a multipoint rather than a single point property of foods.

2.7. Sensory evaluation

Sensory analysis of seafood has become popular in marketing research, product development, quality assurance and research and development. The oldest and still the most widespread means of evaluating the acceptability and edibility of fish are the senses –smell and sight, supplemented by taste and touch (Farber, 1965). Thus sensory evaluation measures the physical properties of food by psychological techniques. It is the subjective taste panel that is used as the standard to determine the accuracy of any objective test (Gould and Peters, 1971). Farber (1965) strongly feels that the line dividing fish that are still fresh from those with some early signs of spoilage is not well defined and is most often subject to difference in personal opinion. Fishery products are having their own characteristic flavour and aroma that are mostly complex in nature, which vary with species and type of treatment applied and which none of the objective methods so far developed can singly bring out successfully. Strong off odours associated with fish spoilage result when metabolites are released by bacterial action (Hebard *et al.*, 1982; Lannelongue *et al.*, 1982 and Reddy *et al.*, 1992). Alternatively, expert

panels trained to assess 'freshness' can discriminate objectively between samples having quite small differences.

Sensory evaluation is still the most reliable method for evaluation of the freshness of raw and processed fishery products (Iyer, 1972). Several investigations have reported a favourable correlation between sensory assessment and the amino nitrogen content of fish, while others questioned the usefulness of amino nitrogen as a measure of early fish spoilage (Farber, 1965). Shewan *et al.*, (1953), Farber (1965) and Mammen (1966) have developed a numerical scoring system for the assessment of freshness. This involved evaluation of condition of eyes, colour of the gills, odour of the gill, general appearance and texture of each intact fish.

Kim and Hearnsberger (1994) evaluated the changes in flavour, odour and appearance of refrigerated catfish fillets in combination with food preservative and / or lactic acid culture. Botta *et al.* (1984) reported that Atlantic cod quality generally did not change appreciably during the first 4-5 days, was moderate during 6-10 days and was poor during 11-13 days of storage and reached rejection state at 15 days of iced storage. Significant losses in flavour and slight toughening were detected in the cooked tissue of cod fillets during frozen storage (Sirois *et al.*, 1991). Kim *et al.* (1995a) evaluated the sensory characteristics of catfish fillets in combination with sodium acetate and monopotassium phosphate and found that the fillets were sensorily acceptable till 12 days at 4°C. Bremner and Statham (1983) reported changes in odours of raw scallops, which were either, air, vacuum packed or

treated with potassium sorbate. Shalini *et al.* (2000) reported that vacuum packed fresh *Lethrinus lentjan* fillets, treated with 2% sodium acetate were sensorily acceptable for 2-3 weeks during refrigerated storage.

2.8. Packaging material

As with other raw meats, fish at retail sale require some form of wrapping to protect the foods from contamination, and the purchase from soiled hands and clothing. An improvement in quality and shelf life of pre-packed fish is obtained depending on the type of fish, packaging material and packaging method. The most important factor in the microbiology of packaged perishable foods like fish is the relative permeability of the packaging material to O_2, CO_2 and water vapour (Adams and Moss, 2000). All packaging films will modify to some extent the gaseous environment of a raw flesh food stored under chill conditions because O_2 is consumed by residual tissue respiration and by metabolic activities of contaminating microorganisms, while CO_2 is produced. Hence, in case of more impermeable films, not only does the O_2 concentration within the pack diminish because atmospheric oxygen is largely excluded but also the CO_2 concentration tends to increase. Although *pseudomonas* and certain other psychrotrophic spoilage organisms are obligate aerobes, their growth is not inhibited until very low oxygen tensions are achieved. For example, Shaw & Nicol (1969) showed that the growth of *pseudomonas* on meat was not affected until the O_2 concentration was reduced below 0.8%, a level unlikely to be achieved in vacuum packs (Ingram, 1962).

As well as having the necessary gas barrier properties, a vacuum packaging film must have mechanical toughness, particularly a high resistance to puncture and abrasion, plus the ability to form reliable seals even in the presence of contamination (e.g. meat / fish juice and fat) and film overlap. The films that best meet all these requirements are composites, which utilize the properties of two or more individual film materials to provide a good package. The composite films are either laminated where the various layers are bonded with an adhesive, or the layers may be co-extruded, or a combination of both lamination and co-extrusion techniques may be involved.

Several studies on vacuum packaging have been carried out using different packaging materials of suitable thickness and oxygen transmission rate. They include, saran coated melinex - polyethylene laminate material (Cann *et al.*, 1965), nylon and low density polyethylene (Huss, 1972), flexible laminate of ethylene vinyl acetate and polyvinyl chloride (Meekin *et al.*, 1982), Cryovac P840 film pouches (Kemp *et al.*, 1989), 50 gauge polyester / 200 gauge polyethylene, Cryovac B - 700, co-extruded nylon / surlyn (Josephson *et al.*, 1985), 100 gauge nylon / 200 gauge copolymer-suryln (Unda *et al.*, 1990), surlyn and saran (Huang *et al.*, 1994) and polyvinyldene chloride films and vacuum skin films (Zhuang *et al.*, 1996). Polyethylene / polyester laminates and nylon - 11 are currently recommended films for vacuum packaging (Anon, 1972).

MATERIALS AND METHODS

3.0. MATERIALS AND METHODS

3.1. Materials

3.1.1. Fish

Pearlspot (*Etroplus suratensis*) and Black Pomfret (*Parastromateus niger*) used for the study were procured from Fort Kochi fish market.

3.1.2. Sample for ice storage

Pearlspot, brackish water fish, caught in the morning by trap fishing and Black pomfret caught by trawl net were used for the study. Condition of raw material of both the species was very fresh with characteristics like convex black pupil, translucent cornea, bright red gills, no bacterial slime, transparent bright opalescent sheen and no bleaching. After washing with clean water, the fish were properly iced with crushed ice in rectangular insulated box and were brought to the laboratory within 20 minutes.

3.1.3. Chemicals and preservatives

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

3.1.4. Packaging material

Pouches (size: 15 × 22 cm) made of 12μ polyester laminated with 300 guage low-density polyethylene were used for packing dressed whole Pearlspot and Black Pomfret steaks. Physical properties of the packaging material used are presented in table 4. Physical properties of the pouch such as tensile

strength and elongation at break were determined in the machine direction and in the cross direction as per IS: 2508 (1984). Heat-seal strength (ASTM, 1972), Oxygen Transmission Rate (ASTM, 1975), Water Vapour Transmission Rate (ASTM, 1987) and Overall Migration Residue for food contact application (FDA, 1983) were also determined.

3.1.5. Packaging material Testing

Before testing all the samples were conditioned at 64%RH at 25±2°C for 24hrs using Gallenkamp Sanyo humidity oven.

3.1.5.1. Determination of Tensile Strength and Elongation at break (IS: 2508-1984)

Tensile strength has been defined as the force parallel to the plane of the specimen required to produce failure in a specimen of specified width and length under specified condition of loading.

Apparatus: Tensile Strength Machine:

The machine used for the measurement maintained a rate of traverse of one grip as constant as possible. The load scale was accurate to within 0.1 N. The load range was such that the breaking load of the test pieces falls between 15% and 85% of the full scale reading.

Samples:

Samples (50±1mm length x 15mm width) were cut in lengthwise and crosswise direction such that the total length was 50mm longer than the gauge length. The thickness was measured using a screw gauge.

The conditioned specimen was clamped between the grips of the machine. Machine was then switched on at a pre adjusted speed of 500 mm/min. The load and elongation at break were recorded. Tensile strength was calculated and expressed as Kg/cm^2. Elongation at break was expressed as percentage of the original length.

3.1.5.2. Determination of Water Vapour Transmission Rate (WVTR) [ASTM E 96-80 (1987)]

It is measured as the quantity of water vapour in gms that will transmit from one side to the other of the film of an area of one sq. meter in 24 hrs. when the relative humidity difference between the two sides is maintained at 90±2% at 37°C.

Apparatus: Test Dishes.

Shallow aluminium dishes of as large a diameter as a can were used. A wax seal between the plastic film (Area-50 cm^2) and the dish was given so as to prevent the transmission of water vapour at or through the edges of the plastic film.

Method:

WVTR was determined by sealing the open end of the dish containing the desiccant (fused Calcium Chloride) by the test specimen and exposing the dish to 90±2% RH at 37°C. Increase in weight of the desiccant after a known period of time was noted from which the WVTR of the film is calculated and expressed as $g/m^2/24hrs$ at 90±2% RH at 37°C.

3.1.5.3. Determination of Gas Transmission Rate: [ASTM (1975) D 1434]

The permeability of plastic films by gases is described as the volumetric rate of transmission of the gas, under known pressure differential, through a known area of film and is usually expressed as the transmission rate in ml per square meter per 24 hrs per atmosphere ($ml/m^2/24$ hrs/atmos). The permeability of plastic materials to different gases is of considerable significance in many applications. It can often be desirable to achieve a certain degree of permeability to certain gases, rather than to produce an entirely impermeable pack.

The phenomenon of gas permeability is dependent on the physical nature of the film, its density, degree of crystallinity and thickness and on the other the size and mobility of the gas molecules. The degree of polarity of both plastic materials and gas molecules as well as their tendency to be either hydrophobic or hydrophilic do influence the permeability of films with respect to particular gases.

Apparatus: Gas Permeability Apparatus (Davenport, U.K.)

Procedure:

The upper half of the permeability cell was removed. As supplied, the apparatus will have the 'X' volume controlling insert correctly fitted in the lower half of the cell. A dried circular filter paper (Whatman No.1) was placed on the top of the insert and the sample of film spread over the filter paper.

The upper part of the film permeability cell was then replaced. The bolts were then reinserted and tightened up with the bo› spanner.

The test gas was now turned on and the cell 'flushed out ' with a brisk stream of gas for a few seconds, after the flow may be reduced to a slow rate, to ensure that no air can diffuse back in to the cell (1 bubble/second through liquid paraffin). The lower part of the cell was then evacuated (using vacuum pump capable of giving a vacuum at least as low as 0.2 mm Hg which can be viewed using a McLeod gauge). The apparatus was tilted to the left until the mercury runs out of the reservoir into the manometer, partially filling it. Returned the apparatus to the normal position and immediately set the movable scale to a convenient starting point, started a stopwatch and began to take readings, at suitable time intervals.

Calculation:

$$GTR = \frac{273xpVx24x10^{4}}{A \times T \times P}$$ where,

GTR = Gas transmission rate in $ml/m^2/24$ hrs at 1 atmosphere pressure difference.

P = Rate of pressure change in capillary in cm. Hg per hour.

V = Total volume in ml of the space between the lower surface of the film and the top of the mercury column in the capillary.

This total volume expressed as,

(a) The volume of cell cavity (i.e. 5 cc)

(b) The volume of capillary tube above the mercury level half way through test; as the area of cross section of the capillary is 0.018 cm^2, this volume will be 0.018 X, when X

is the length of the capillary above the mercury at the half way point in cm.

(c) The 'free space' volume of filter paper - can be taken as 0.24 ml.

A = Area of the specimen - 23.77cm^2

T = Temp. in °K (273+°C)

P = Pressure difference (76cm Hg)

i.e. = $\frac{273 \times pV \times 24 \times 10^4}{23.77 \times 76\ (273+°C)}$

3.1.5.4. Determination of Overall Migration Residue (FDA, 1983)

Migration is the mass transfer of materials from plastics to foods under specified conditions. Migrants are materials thus transferred from plastics to food. In order to assess the toxic effects of the plastic packaging materials, the specification laid by various countries prescribe short-term extraction test called the global migration tests for quantifying the migrants. It is difficult to estimate quantitatively the amount of migrants in actual foodstuffs because of the complex nature of the food and diverse food categories. Accordingly, global migration tests, measure gravimetrically the amount of migrants from plastics to different Food Simulating Solvents (FSS), which are based on the Categorisation of foods (Table. 1).

Table: 1. Food Categorisation: - As per FDA (1993)

I	Non-acid, aqueous products, may contain salt or sugar or both (above pH 5)
II	Acid, aqueous products, may contain salt or sugar or both including oil-in-water emulsions of low or high fat content (pH below 5)
III	Aqueous, acid at non-acid products containing free oil or fat, may contain salt and including water-in-oil emulsions of low or high fat content.
IV	Dairy products and modifications A. Water-in-oil emulsions, high or low fat B. Oil-in-water emulsions, high or low fat.
V	Low moisture fats and oils.
VI	Beverages: A. Containing upto 8 % alcohol B. Non-alcoholic C. Containing more than 8 % alcohol
VII	Bakery products A. Moist bakery products with surface containing free fat or oil B. Moist bakery products with surface containing no free fat or oil
VIII	Dry solids with the surface containing no free fat or oil (no end test required)
IX	Dry solids with the surface containing free fat or oil

Method:

Filled the pouch to their filled capacity with glass distilled water at test temperature (21.1°C) and exposed for 48 hrs at this temperature. After

exposure, the pouch was removed quickly and the distilled water was transferred into clean glass beaker with three washing using glass distilled water.

The extractant was evaporated to about 50-60 ml and transferred into a clean tared stainless steel dish along with three washings and further evaporated to dryness in an oven at 100°C. The dish was then cooled in a dessicator for 30 minutes and weighed. Calculated the extractives in milligrams/dm^2 or mg/litre.

$$OMR = \frac{\text{Mass of residue in mg} \times 100}{\text{(A) Area exposed in } cm^2} \quad mg/dm^2 \text{ or}$$

$$= \frac{\text{Mass of Residue in mg} \times 1000}{\text{(V) Volume of simulant in ml.}} \quad \text{mg/litre (ppm)}$$

3.1.6. Vacuum sealing machine: Vacuum sealing machine (Model QS 400VD) (Plate: 1) supplied by M/s. Sevana Electrical Appliances Pvt. Ltd., Kizhikkambalam, Kerala, was used for packing the fish steaks.

3.1.7. Bacteriological media: Dehydrated bacteriological media such as Plate Count Agar (PCA) (BBL Difco), Baired Parker Agar (BP, Himedia), Kenners Faecal (KF) Agar (BBL Difco), de Man Rogosa and Sharpe (MRS) Agar (Himedia, India), Cetrimide Fucidin Cephaloridine (CFC) Agar (Oxoid) and Violet Red Bile Glucose Agar (VRBGA, Oxoid), Peptone Iron Agar (BBL Difco) and Tryptose Sulphite Cycloserine (TSC) Agar (Himedia, India) were used.

Plate. 1. Vacuum Sealing Machine

3.1.8. Diluent: Physiological saline (0.85% NaCl) was used as diluent for all microbiological assays.

3.1.9. Chlorine water: Dressed Pearlspot and Black Pomfret steaks were given a dip treatment for 10 min in 2 ppm chilled chlorinated water.

3.2. Methods:

3.2.1. Preparation of fish samples: Fresh Pearlspot and Black Pomfret were brought to the laboratory in iced condition. The fish were washed in fresh water and kept in iced condition during processing. Both the species were beheaded, scaled, gutted and washed in potable water. Dressed fish was used as such in case of Pearlspot, whereas in case of Black Pomfret dressed fish was cut into steaks and again washed in potable water.

3.2.2. Methods of treatment

The treatment solutions of sodium acetate and potassium sorbate were prepared in potable water. Dressed whole Pearlspot / black pomfret steaks were divided into four lots and subjected to packaging with or without preservative treatment as detailed below:

Lot I : Untreated fish/steaks were packed without vacuum(air pack)

Lot II : Untreated fish/steaks were vacuum packed.

Lot III : Fish/steaks were dip treated in sodium acetate solution for 30 minutes and then packed after draining the solution.

Lot IV : Fish/steaks were dip treated in potassium sorbate solution for 30 minutes and then packed after draining the solution.

Samples were coded as follows:

Lot No.	Description	Code
I	Control air pack	CAP
II	Control vacuum pack	CVP
III	Sodium acetate treated vacuum pack	SAVP
IV	Potassium sorbate treated vacuum pack	PSVP

3.2.3. Packing:

Fish/steaks (200±5 g) were placed in each laminated pouch having the configuration of 12μ Polyester laminated with 300 gauge low density polyethylene. Lot I (air pack) was sealed using a heat sealer. Remaining Lots (II, III & IV) were vacuum packed at –1 bar pressure.

3.2.4. Ice storage

3.2.4.1. Icing:

Immediately after packing, all the packs were iced with flake ice in the ratio of (1:1) fish: ice in an insulated box. A thick layer of ice was put at the bottom, then fish/steaks packs and ice were put alternatively and the packs were covered on top with a layer of ice. Care was taken to cover all the packs with ice. Insulated fish box was kept in a chill room maintained at 0–2°C. Re-icing was done everyday to supplement the loss due to melting, after draining the melted ice.

3.2.4.2. Sampling:

Samples were drawn from each lot at regular inte vals for analysis. All the packs were analysed for sensory, microbiological, textural and biochemical parameters. Sampling was done in triplicate and the mean values were taken.

3.2.5. Standardisation of concentration of sodium acetate/potassium sorbate

To standardize the concentration of sodium acetate and potassium sorbate for dip treatment, solutions of different concentrations (1%, 1.5%, 2% & 2.5% w/v) of both were prepared in potable water. Dressed pearlspot/black pomfret steaks were washed thoroughly in chilled water and a dip treatment in 2 ppm chilled chlorine water was given for 10 minutes. Fish were drained well and divided into 10 lots each. One lot of each of the species was subjected to air packing and the second lot was subjected to vacuum packing. The other 8 lots of each of the species were given a dip treatment in sodium acetate/potassium sorbate solutions of different concentrations (1%, 1.5%, 2% & 2.5% w/v) for 30 minutes, drained well and vacuum packed. After packing the pouches were immediately transferred to insulated boxes with proper icing. The box was kept in a chill room till the end of storage. Sampling was done in triplicate and the mean value was taken. Ideal concentration was selected based on sensory evaluation.

3.3. Biochemical analysis

3.3.1. Determination of Moisture:

Moisture content of the fish was determined according to AOAC (2000). About 10 g of minced fish meat was weighed into a clean and dry, pre-weighed petridish. The dishes were kept open in hot air oven at 100 ± 1°C for 16-18 hrs. The same were then cooled in desiccators and weighed, till constant weights were obtained. Moisture content in the sample was calculated and expressed as percentage.

3.3.2. Determination of crude protein:

The total nitrogen present in the sample was determined according to AOAC (2000). About 0.5 – 1g of sample was accurately weighed and taken in a clean and dry kjeldahl digestion flask. A pinch of digestion mixture (8 parts potassium sulphate and 1 part copper sulphate) and 10 ml of concentrated sulphuric acid were added to the digestion flask. A few glass beads were added to avoid bumping. The flask was heated on micro digestion unit until the sample became clear and colourless. Distillation was done in a micro kjeldahl distillation unit. The distillate was collected in 10 ml of 2% boric acid solution and the nitrogen content in the sample was estimated by titrating against 0.01 N standard sulphuric acid. The crude protein in the sample was obtained by multiplying total nitrogen content in the sample with the conversion factor 6.25.

3.3.3. Determination of Ash:

Determination of ash content of the fish meat was done according to AOAC (2000). About 2 g of the moisture free sample was weighed into a porcelain crucible and was heated over a low flame till all the materials got completely charred. The charred sample was heated in a muffle furnace for about 3 – 5 hours at 600°C until the ash turned white or grayish white in colour. The sample was cooled, weighed and the ash content was calculated and expressed as percentage.

3.3.4. Estimation of crude fat:

Determination of crude fat of the fish meat was done according to AOAC (2000). About 2 – 3 g of accurately weighed moisture free sample was taken in a thimble plugged with cotton and was extracted with petroleum ether (40 – 60°C boiling point) in a Soxhlet apparatus for about 10 h at a condensation rate of 5–6 drops per sec. Excess solvent was evaporated and the fat was dried at 100°C to constant weight. The crude fat was calculated and expressed as percentage.

3.3.5. Drip loss:

Drip loss was measured according to Randell *et al.* (1997). Raw fish was removed from the packages and the mass of drip left in the package (g) was divided by the mass of the product (g). Drip loss was expressed as percentage.

3.3.6. Determination of pH:

pH of the fish meat samples were determined according to APHA (1998). 10 g of the minced fish were blended with 10 ml of distilled water and pH was measured using a digital pH meter (Cyberscan 510, UK).

3.3.7. Preparation of Tri Chloro Acetic acid (TCA) extract:

About 10 g of accurately weighed sample was extracted with 10% trichloro acetic acid (TCA) by grinding in a mortar and pestle, then the content was filtered quantitatively through Whatman Filter paper No.1. Filter paper was thoroughly washed with TCA and filtrate was made up to 100 ml. The TCA extract was used to measure Trimethyl Amine Nitrogen and Total Volatile base Nitrogen of fish.

3.3.8. Determination of total volatile base nitrogen (TVB-N):

Total volatile bases in the samples were determined as total volatile base nitrogen (TVB-N) by the micro diffusion method (Conway, 1950). 1 ml of standard N/100 sulphuric acid was taken in the inner chamber of the diffusion unit. To the outer chamber 1 ml of TCA extract was added followed by 1 ml of saturated potassium carbonate. The unit was then sealed with the glass lid and kept undisturbed overnight. The amount of unreacted acid in the inner chamber was determined by titrating against standard N/100 sodium hydroxide with Tashiro's indictor. Similarly a blank was also run. TVB-N was calculated and expressed as mg $100g^{-1}$ of the sample.

3.3.9. Determination of Trimethylamine (TMA):

TMA was determined as trimethyl amine nitrogen (TMA-N) by the micro diffusion method (Conway, 1950). 1 ml of standard N/100 sulphuric acid was taken in the inner chamber of the diffusion unit. To the outer chamber 1 ml of TCA extract was taken followed by 1 ml neutralized formaldehyde. This was kept as such for 3 min. to ensure that formaldehyde had bound with all the primary and secondary amines and ammonia contained in the extract. The analysis was further carried out as explained in TVB-N determination. TMA-N was calculated and expressed as mg $100g^{-1}$ of the sample.

3.3.10. Determination of Thiobarbutric Acid (TBA) Value:

About 10 g of fish meat was mixed with 100 ml 0.2 N HCl and homogenized to slurry. This slurry was then poured in to a round bottom flask connected to the TBA distillation apparatus. Distillation was done until 50 ml of the distillate is collected within 10 minutes. 5 ml of distillate was taken in a test tube and 5 ml of TBA reagent was added and heated for 35 minutes. A blank was also done with distilled water. Colour developed was measured in a spectrophotometer at 538 nm and TBA value was determined and expressed as mg malonaldehyde/kg of fish sample (Tarladgis *et al.*, 1960).

3.3.11. Determination of K value:

The nucleotide and related compounds in the muscle (taken from the dorsal region) were determined by the method of Ryder (1985) using High Performance Liquid Chromatography. A Merck system was used, with a

bonda packed C18 stainless steel column. Extraction of nucleotide from muscle was done using 0.6M Perchloric acid at 0°C and neutralized usir g 1M KOH. It was then filtered through a Millipore (0.45μm) syringe filter. Nucleotide standards and potassium phosphates were obtained from Sigma Chemical Company. Mobile phase comprised of 0.06 M K_2HPO_4 and 0.04M KH_2PO_4 at pH 6.5-6.8. Buffer solutions were prepared daily in Milli Q water and filtered through a millipore filter (0.45μm). The flow rate was 1.5 ml/min. and the eluate was monitored at 254nm. The detector response for each of the six nucleotides found in fish muscle was calibrated daily by injecting 20μl of 0.166mM solution of each reference compound. All solutions were passed through a 0.45μm aqueous filter before injection onto the column. The K-value was computed from the results as defined by Saito *et al.* (1959).

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

3.4. Texture Profile Analysis

Texture profile analysis was measured with a Universal Testing Machine (Lloyd instruments LRX plus, UK) as described by Anderson *et al.* (1994). The main part of the instrument was a load cell. Standard cells (5000 N, 500 N and 50 N) can be used depending on the type. The LRX plus machine was fitted with two magnetically activating limit stops. Reaching magnetically activated limit stop will result in the machine stopping. The speed of the

cross edge movement varies from 0.01-1016 mm/min. The unit has a liquid crystal display (LCD) to show set up information, load and extension values and a key pad to input information for operating the machine when under the control of the console. Texture profile analysis was performed on uniform samples, which were allowed for a compression of 40% with a trigger force of 0.5 kg. Cooked fish pieces (uniform samples boiled in 1.5% brine for 10min., drained well and cooled) were compressed twice by cylindrical probe having a diameter of 50mm, at a test speed of 12mm/min equipped with a 50N load cell. The principle of cylindrical probe is that, as the probe is forced into the specimen, a shearing force acts which causes the sample to deform or rupture. This produces a curve showing load resulting from deformation. Cylindrical probes have traditionally been used as general purpose compression probes for a wide range of applications, primarily for applying a deformation to a gel structure to determine the gel strength and elasticity. They are also useful for applying a shearing force to a gel to determine its breaking strength and elasticity. Many standards quote cylindrical probes for gel testing. Hardness, cohesiveness, springiness and chewiness were calculated as defined in the texture analyzer user manual. Texture Profile Analysis results was tabulated using Nexygen Software.

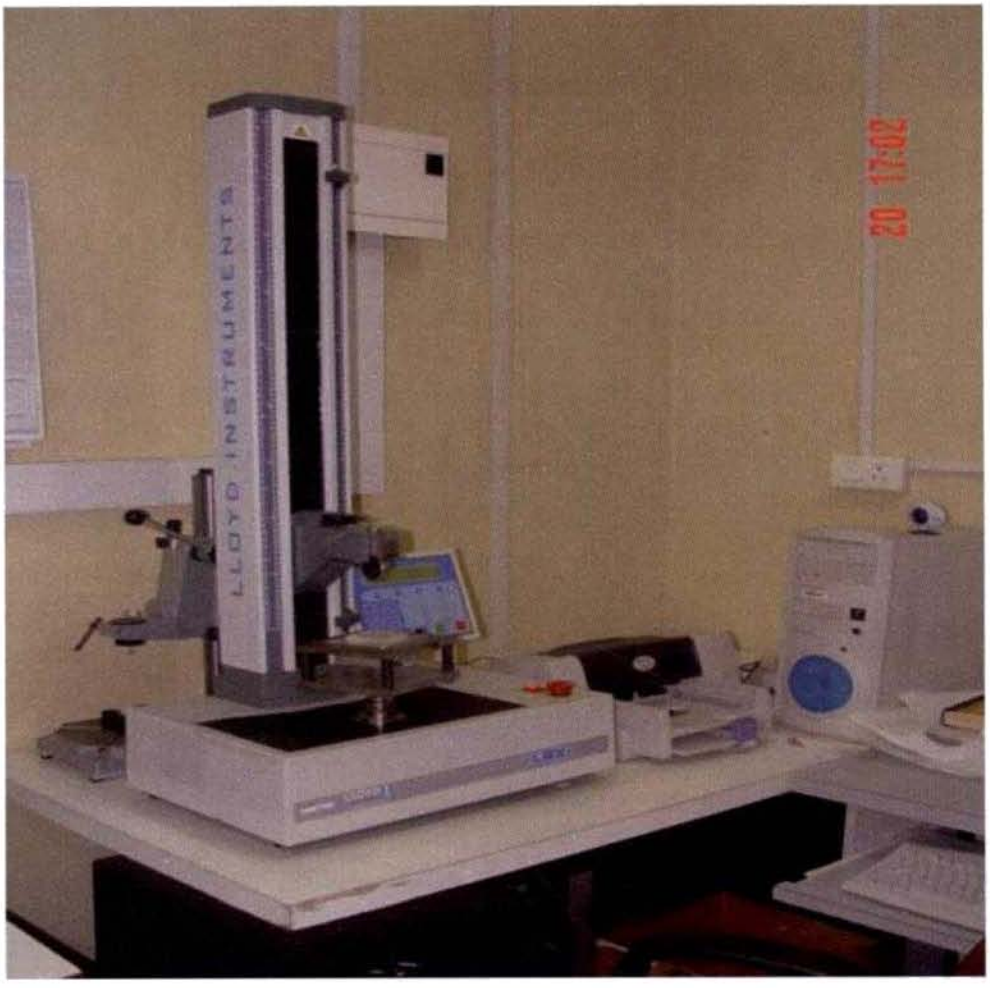


Plate: 2. Food Texture Analyser

3.5. Microbiological Analysis

3.5.1. Preparation of sample for bacteriological analysis

Twenty five gram of fish was aseptically weighed and homogenized with 225 ml sterile 0.85 % normal saline for one min. in Stomacher 400 lab blender (Seward medical, London, UK). The homogenized sample was serially diluted using sterile 9 ml saline for bacteriological analysis. Counts of *Staphylococcus aureus, Faecal Streptococci* and *Enterobacteriacea* were determined for fresh fish and for fish at the time of sensory rejection. Total viable counts (TVC), counts of H_2S producing bacteria, *Pseudomonas*, Lactic acid bacteria and sulphite reducing clostridia were also determined.

3.5.2. Total Viable Count (TVC)

Total viable counts were determined in Plate Count Agar (code 247940, BBL Difco) by spread plate method (AOAC, 2002). 0.5 ml of each dilutions viz. 10^3, 10^4, 10^5 and 10^6 of the samples prepared were added to four predried sterile Plate Count Agar plates. After spreading using sterile bent glass rod, the plates were incubated at 20°C for 4 -5 days and at 37°C for 48hrs. After incubation, all the colonies developed on the agar were counted using Quebec colony counter and the average count of the duplicate plates were recorded. Counts were expressed as colony forming units of total aerobic bacteria (cfu/g).

3.5.3. Hydrogen sulphide (H_2S) producing bacterial count

Hydrogen sulphide produ :ing bacteria were enumerated on Peptone Iron agar (code 289100, BBL Difco) plates after incubation at 20°C for 5 days (Gram *et al.*, 1987). 0.5 ml of appropriate dilutions of sample was added in duplicate to pre-dried sterile IA plates and spreaded using sterile bent glass rod. After spreading, the plates were overlaid with the same medium and incubated at 20°C for 5 days. Black colonies formed by the production of H_2S were enumerated (Gennari and Campanini, 1991) and the counts were expressed as number of colony forming units of H_2S producing bacteria per gram of the sample (cfu/g).

3.5.4. Pseudomonas

For the enumeration of *Pseudomonas*, Cetrimide Fucidin Cephaloridine agar (CFC, Oxoid code CM 559 supplemented with SR 103) was used as per Mead and Adams (1977). Samples (0.5 ml) of appropriate serial dilutions of fish homogenates were added in duplicate to pre-dried sterile CFC plates and spreaded using sterile bent glass rod. The plates were then incubated at 20°C for 2 days. To the incubated plates cytochrome oxidase reagent was added and the colonies, which retain the blue colour were counted as *Pseudomonas*. Counts were expressed as number of colony forming units (cfu) of *Pseudomonas* per gram of fish.

3.5.5. Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) were enumerated in MRS Agar (de Man, Rogosa and Sharpe, Himedia code M641) supplemented with Potasssium sorbate as per Mossel (1987). Samples (0.5 ml) of appropriate serial dilutions of fish homogenate were added in duplicate to pre-dried sterile MRS agar plates and spreaded using sterile bent glass rod. After spreading, plates were overlaid with the same medium and incubated at 37°C in a Carbon dioxide incubator at a CO_2 level of 5%, for 48-72 hrs. Pure white colonies (2-3mm dia) were counted as LAB. The average count of duplicates was calculated and expressed as colony forming units (cfu) of LAB per gram of fish.

3.5.6. *Enterobacteriaceae*

Members of the family *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose Agar (VRBGA, Oxoid, CM 485) as per Koutsoumanis and Nychas (1999). One ml of appropriate serial dilutions of fish homogenate was inoculated into 15 ml of molten VRBGA medium cooled to 45°C, mixed well and allowed to set for 15 min. The plates were incubated at 30°C for 24 hrs. Large colonies with purple haloes were counted as *Enterobacteriaceae*. The average counts of duplicates were calculated and expressed as colony forming units of *Enterobacteriaceae* per gram (cfu/g) of the sample.

3.5.7. *Faecal Streptococci*

Members of *Faecal streptococci* were enumerated on Kenner Faecal Agar (KF, BBL Difco code 249160) after incubation at 37°C for 36-48 hrs (USFDA,

1995). One ml of appropriate serial dilutions of fish homogenate was pipetted to sterile petridish taken in duplicate for each dilution. About 15-18ml of molten Kenner Faecal Agar cooled to 45°C was poured to each plate, mixed well and allowed to set for 30 min. The plates were incubated at 37°C for 36-48 hrs. All red to pink colonies were counted as *Faecal Streptococci.* The average count of duplicates were calculated and were expressed as colony forming units of *Faecal Streptococci* per gram (cfu/g) of the sample.

3.5.8. *Staphylococcus aureus*

Staphylococcus aureus was enumerated on Baird Parker Agar (BP, Himedia code MM043) after incubation at 37°C for 36-48 hrs (AOAC, 2002). 0.5 ml of appropriate serial dilutions of fish homogenate were added in duplicate to pre-dried sterile Baird Parker agar plates. Then it was spreaded using sterile bent glass rod. The plates were incubated at 37°C for 36-48 hours. The colonies of *Staphylococcus aureus* were black, convex, with entire margin surrounded by a clearing zone of 2-5 mm width. Typical colonies were isolated and checked for coagulase enzyme and coagulase positive colonies were counted. Counts were expressed as number of colony forming units of *Staphylococcus aureus* per gram (cfu/g) of the sample.

3.5.9. Sulphite reducing clostridia

Sulphite reducing clostridia were enumerated on Tryptose Sulphite Cycloserine Agar (TSC, Himedia code M837) supplemented with D-Cycloserine after anaerobic incubation (Oxoid Gas Pak Anaerobic jar) at 30°C (Mead *et*

al., 1982). One ml of appropriate serial dilutions of fish homogenate was pipetted to sterile petr dish taken in duplicate for each dilution. About 15-18 ml of molten Tryptose Sulphite Cycloserine agar cooled to 45°C was poured to each plate, mixed well and allowed to set for 30 min. After setting, plates were overlaid with the same medium and again allowed to set. The plates were incubated anaerobically at 30°C for 48 hrs. Black colonies were counted as sulphite reducing clostridia. Typical colonies were checked for stormy clot reaction in Iron milk medium. Colonies showing positive reaction were counted as sulphite reducing clostridia and expressed as colony forming units of sulphite reducing clostridia per gram (cfu/g) of the sample.

3.5.10. *Clostridium botulinum* toxin detection by mouse bioassay

C. botulinum toxin was detected by mouse bioassay as per the procedure described in U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA, 2001). An extract of the sample was made by homogenizing the sample with a suitable volume (1:2 or 1:3) of gelatin phosphate buffer (pH 6.2). Centrifuged at 10000g for 10 min at 4°C and the supernatant was tested for botulinum toxin by mouse bioassay. A portion of the supernatant was diluted to 1:5 in gelatin-phosphate buffer and divided into three. One aliquot was treated with trypsin after adjusting the pH to 6.2 with 1N NaOH or HCl {added 0.2 ml of trypsin (Difco) 1:250, 10% solution to 1.8 ml diluted supernatant} and incubated at 37°C for 60 min. Second aliquot was heated for 10 min in a boiling water bath and cooled and the third aliquot was kept untreated. Injected separate pairs of Webster mice weighing 18-26 g

intraperitoneally with 0.5 ml of each of the three aliquots (untreated, trypsinised and heated supernatant) using a 2 ml sterile syringe with a, 5/8 inch 25 gauge needle, leaving a pair of mice as control.

All mice were observed periodically for 96 hrs for symptoms of botulism and death. Typical botulism signs in mice are ruffling of fur, followed in sequence by laboured breathing, weakness of limbs and finally total paralysis with gasping for breath, followed by death due to respiratory failure.

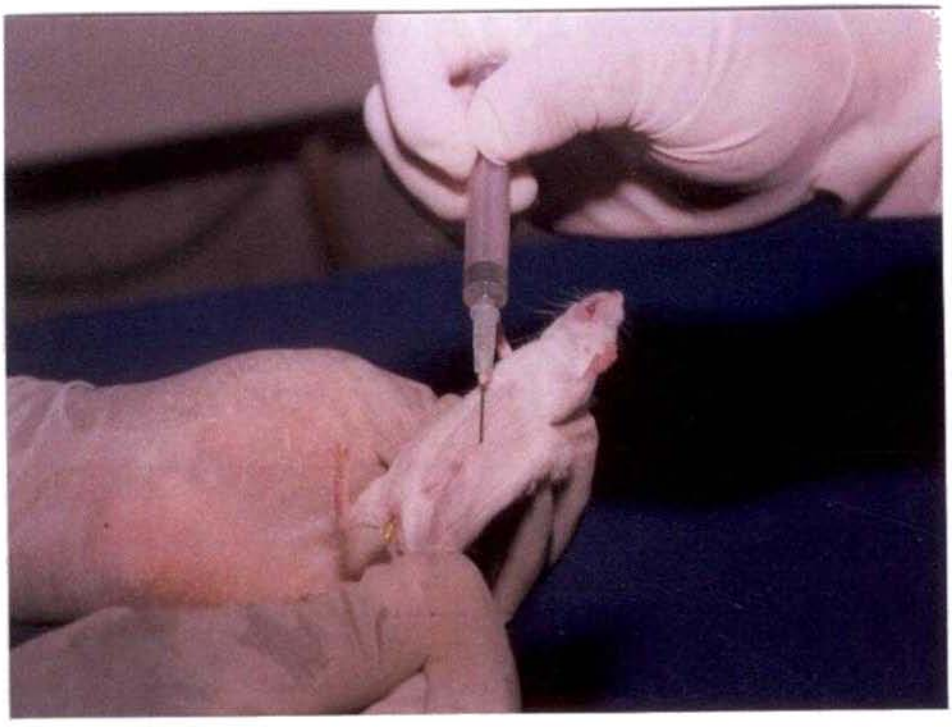


Plate: 3. Mouse bioassay

3.6. Sensory Evaluation

Sensory evaluation was based on characterization and differentiation of the various sensory characters such as appearance, texture, odour and flavour. Sensory analysis was done after cooking the fish samples in 1.5% brine for 10 min. Score was given based on 9-point hedonic scale (Table. 2), as described by Amerine *et al.* (1965). A sensory score of 4 was taken as the borderline of acceptability.

Table: 2. Sensory scores for taste panel studies

Observation (Cooked sample)	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

RESULTS AND DISCUSSION

4.0. RESULTS AND DISCUSSION

4.1. Proximate composition

Proximate composition of fresh Pearlspot analysed had 77.75 % moisture, 2.04 % crude fat, 19.23 % crude protein and 0.98 % ash and of fresh Black Pomfret analysed had 75.92 % moisture, 2.48 % crude fat, 20.04 % crude protein and 1.56 % ash (Table 3).

Table 3: Proximate composition of fresh Pearlspot and Black Pomfret

Component	Pearlspot	Black Pomfret
	(%)	(%)
Moisture	77.75	75.92
Crude fat	2.04	2.48
Crude protein	19.23	20.04
Ash	0.98	1.56

4.2. Physical properties of the packaging material:

Physical properties of the packaging material used are given in table 4.

Table 4: Physical properties of the packaging material

Physical properties	Value
Tensile strength – MD	363 Kg/cm^2
Tensile strength – CD	349 Kg/cm^2
Elongation at break - MD	80 %
Elongation at break –CD	80 %
Heat Seal Strength - MD	249 kg/cm^2
Heat Seal Strength – CD	194 kg/cm^2
Water Vapour transmission rate	3.62 g/m^2/24h at 37°C and 90±2% RH
Oxygen transmission rate (OTR)	65cc/m^2/atmosphere/24hrs at room temperature 28-32°C

MD – Machine Direction
CD – Cross Direction

Packaging material used for the study has got enough strength to withstand machine handling. The overall migration residue (water extractives) at 21.1°C for 48 hrs of the sample was 3.35 mg/l, which is below the acceptable limit for food contact application. It also exhibited lower water vapour transmission rate and oxygen transmission rate that suits for packing vacuum packed products.

4.3. Chemical and Microbiological quality of fresh pearlspot and black pomfret

The chemical and microbiological quality of fresh pearl spot and that of fresh black pomfret are given in Table 5. It is seen from the table that, both the species used for the study had low initial values for TBA, TVB-N, and TMA-N. The results indicate that the quality of raw material of both the species was very fresh.

Table 5: Chemical and microbiological quality of fresh pearl spot and black pomfret

Parameter	Initial value	
	Pearlspot	**Black Pomfret**
TBA	0.08 mg malonaldehyde/ kg of fish	0.28 mg malonaldehyde/ kg of fish
TVB-N	5.6 mg%	4.5 mg%
TMA	1.4 mg%	2.8 mg%
K value	4.87 %	7.81 %
TVC (20°C)	4.98 $\log_{10}$cfu/g	5.48 $\log_{10}$cfu/g
Staphylococcus aureus	1.20 $\log_{10}$cfu/g	1.4 $\log_{10}$cfu/g
Faecal Streptococci	2.58 $\log_{10}$cfu/g	3.08 $\log_{10}$cfu/g
Enterobacteriaceae	2.81 $\log_{10}$cfu/g	2.9 $\log_{10}$cfu/g

4.4. Standardisation of concentration of sodium acetate and potassium sorbate

Standardization of concentrations (1%, 1.5%, 2% and 2.5% w/v) was based on sensory analysis. Sensory analysis was done on a 9 point hedonic scale and a score of 4 was taken as the limit of acceptability. Changes in the sensory scores of pearlspot samples treated with sodium acetate and potassium sorbate of different concentrations in comparison to air and vacuum packed samples are shown in Figures 1 and 2 respectively.

The odour of fish (evaluated as odour when opening the package) changed from neutral odour to off odours once spoilage had taken place during chill storage. Air stored fish were sensorily rejected after 8 days of storage. CVP, SAVP (1%) and PSVP (1%) samples were acceptable up to 10 days. As the days of storage in ice progressed, the sweet taste of the muscle was lost and the texture became soft and pasty. Thus, SAVP (1.5%), SAVP (2.5%), PSVP (1.5%) and PSVP (2.5%) remained in acceptable condition only up to 12 days. Whereas SAVP (2%) and PSVP (2%) samples had a better sensory score compared to other packs and remained in good and acceptable condition up to 15 days. So 2% concentration of sodium acetate and potassium sorbate was used for further detailed studies.

Fig: 1. Changes in overall sensory scores of pearlspot packed under air and vacuum (sodium acetate treated and untreated) during storage at 0-2°C

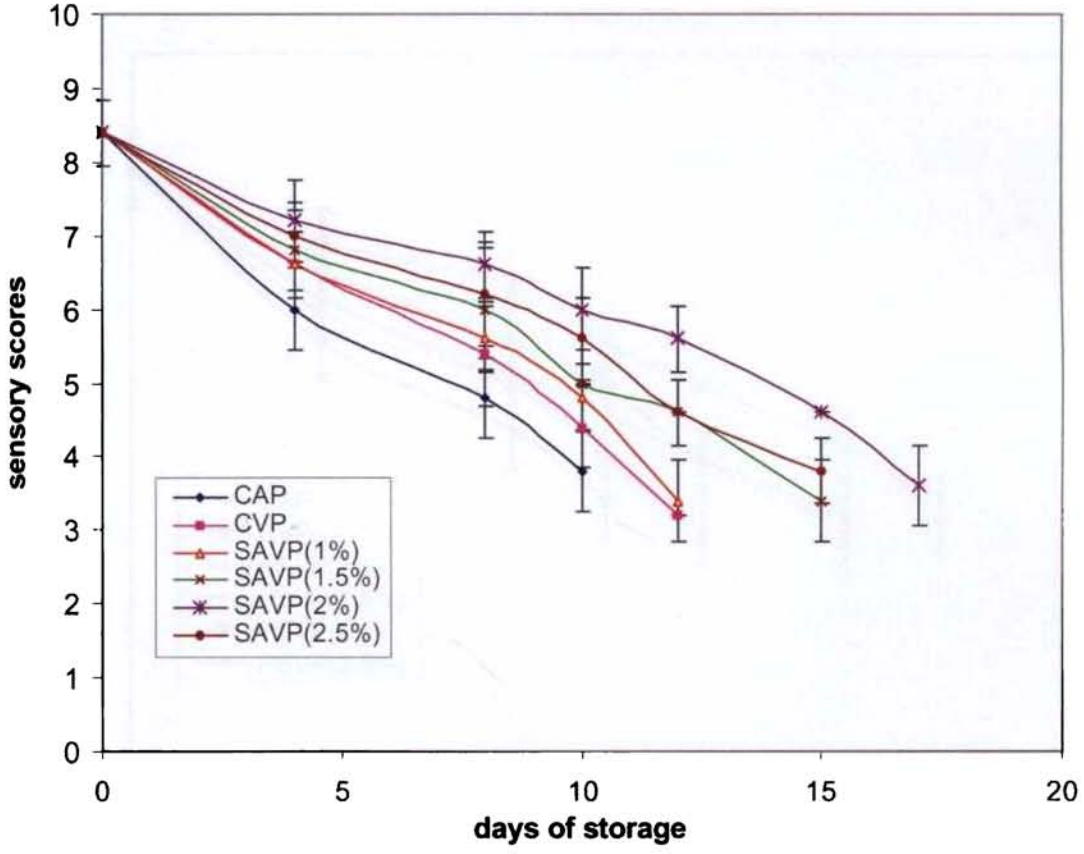
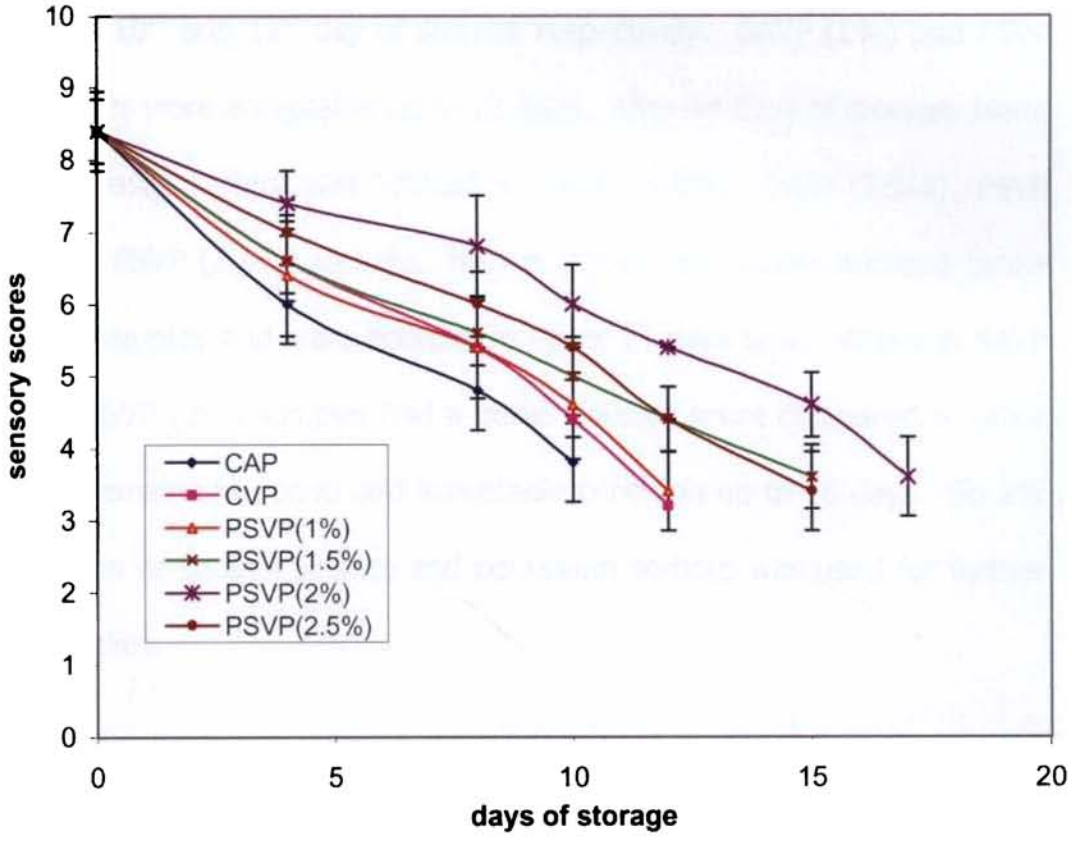


Fig: 2. Changes in overall sensory scores of pearlspot packed under air and vacuum (potassium sorbate treated and untreated) during storage at 0-2°C



Changes in the sensory scores of Black pomfret samples treated with sodium acetate and potassium sorbate of different concentrations in comparison to air and vacuum packed samples are shown in Figures 3 and 4 respectively. Fish spoilage gave rise to the subsequent development of strongly fishy, rancid and putrid odours, and fish was clearly rejected for consumption by the taste panel. Sensory score declined from initial 8 to 3.4 and 3 in CAP and CVP samples on 10^{th} and 12^{th} day of storage respectively. SAVP (1%) and PSVP (1%) samples were acceptable up to 12 days. After 14 days of storage, bland taste and pasty texture was noticed in SAVP (1.5%), SAVP (2.5%), PSVP (1.5%) and PSVP (2.5%) samples. Hence, the sensory scores declined below 4 in these samples and were acceptable up to 14 days only. Whereas SAVP (2%) and PSVP (2%) samples had a better sensory score compared to other packs and remained in good and acceptable condition up to 16 days. So 2% concentration of sodium acetate and potassium sorbate was used for further detailed studies.

Fig: 3. Changes in overall sensory scores of black pomfret packed under air and vacuum (sodium acetate treated and untreated) during storage at 0-2°C

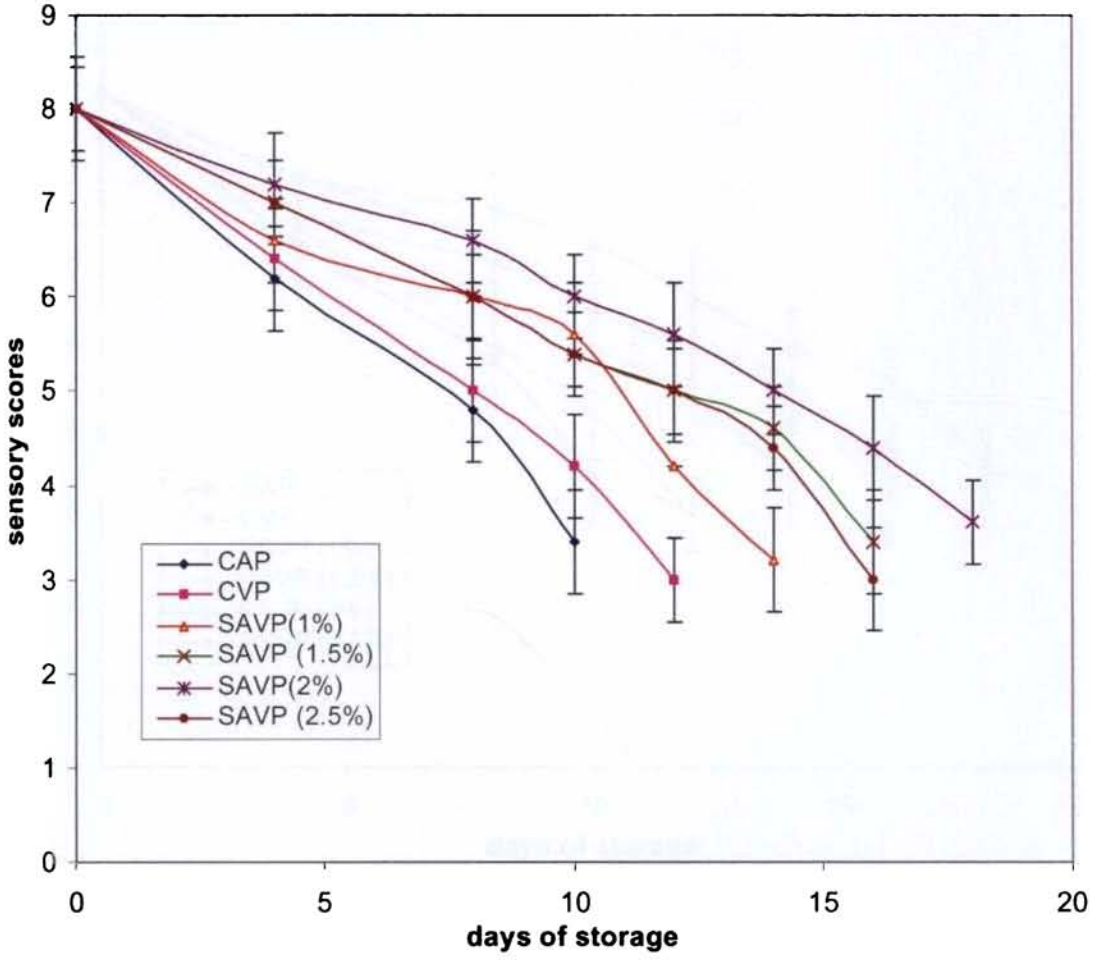
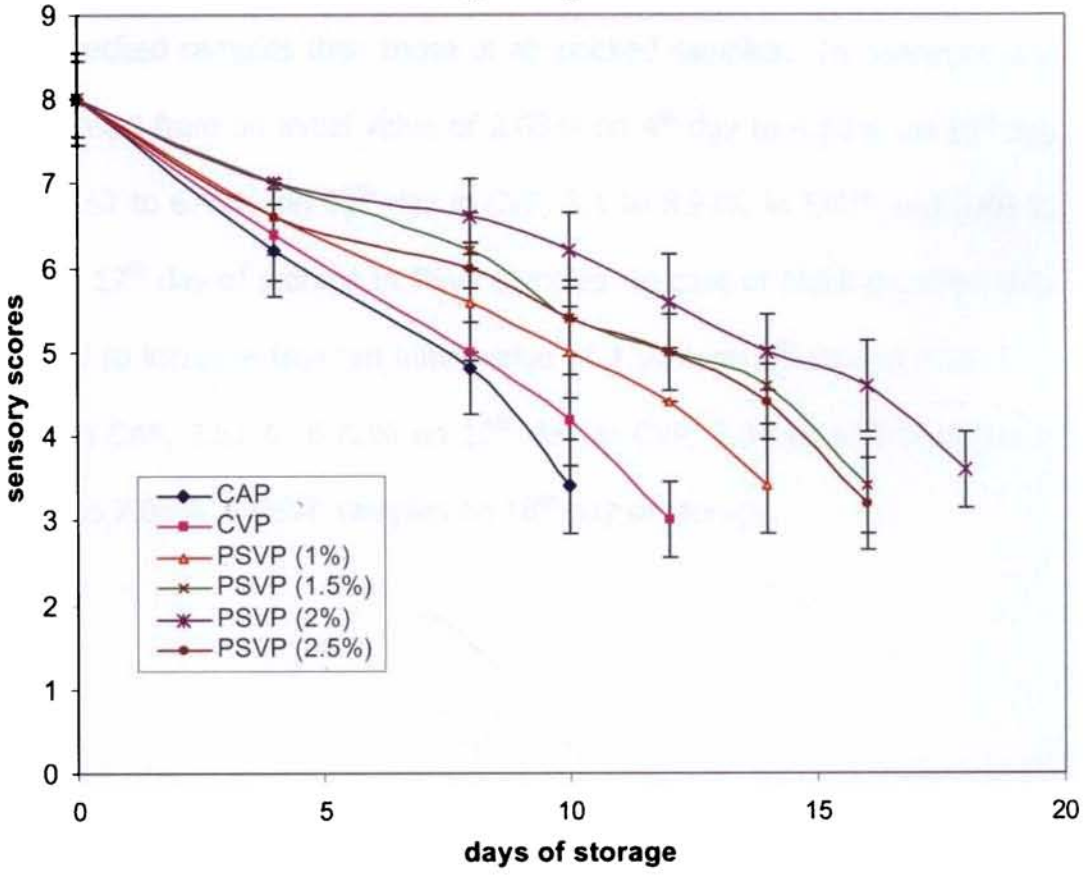


Fig: 4. Changes in overall sensory scores of black pomfret packed under air and vacuum (potassium sorbate treated and untreated) during storage at 0-2°C

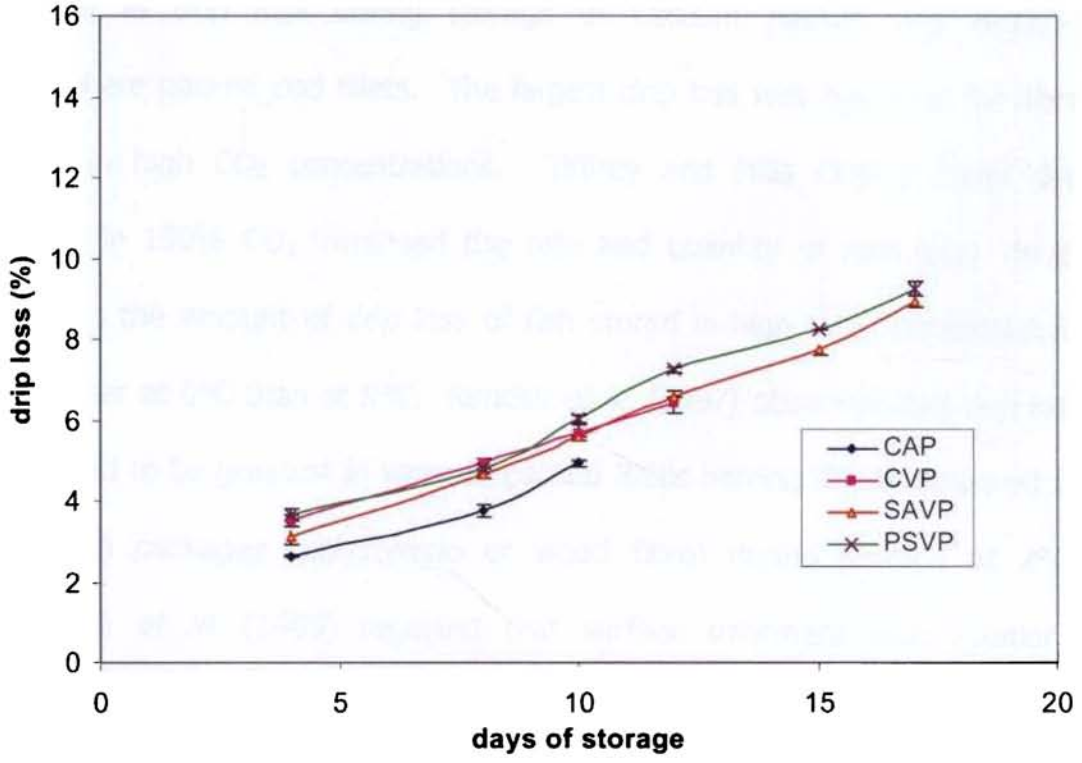


4.5. Biochemical changes

4.5.1. Changes in drip loss during chill storage

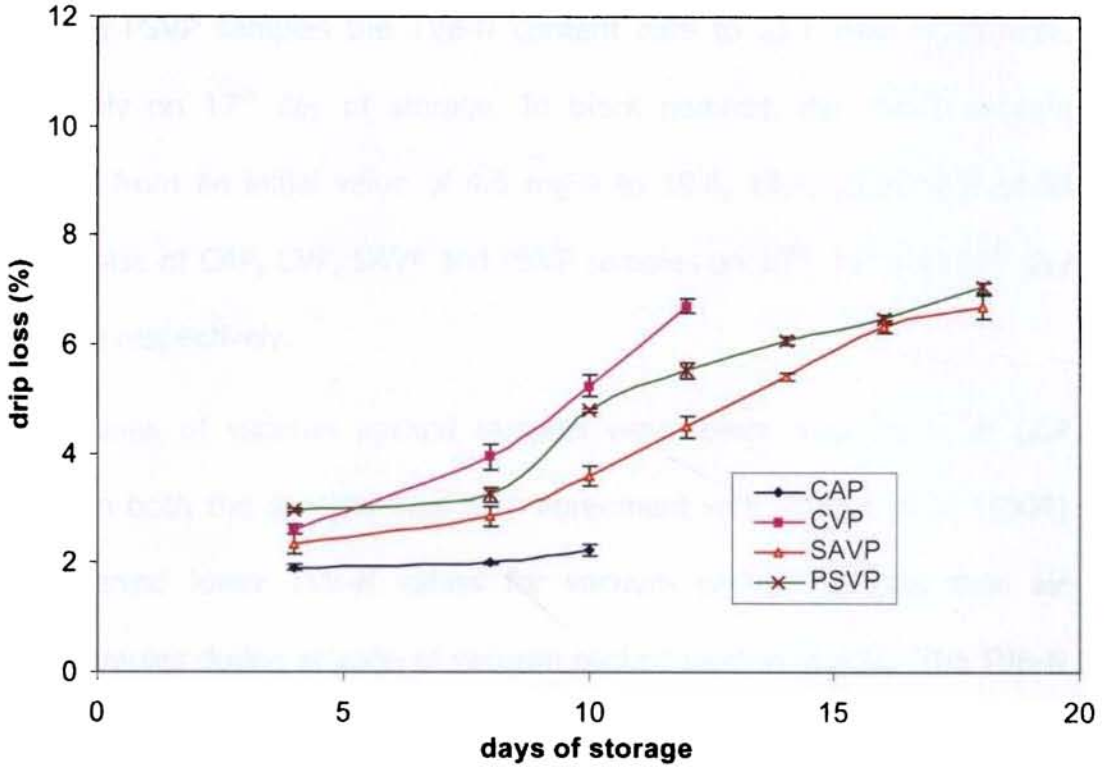
The changes in drip loss values of pearlspot and black pomfret during chill storage are shown in figures 5 and 6 respectively. In both the species an increase in drip loss was observed in case of control and treated packs during chill storage. Drip loss was found to be comparatively more in case of vacuum packed samples than those of air packed samples. In pearlspot drip loss increased from an initial value of 2.63% on 4^{th} day to 4.92% on 10^{th} day in CAP, 3.52 to 6.43% on 12^{th} day in CVP, 3.1 to 8.92% in SAVP and 3.66 to 9.25% on 17^{th} day of storage in PSVP samples. In case of black pomfret, drip was found to increase from an initial value of 1.92% on 4^{th} day to 2.23% on 10^{th} day in CAP, 2.61 to 6.71% on 12^{th} day in CVP, 2.34 to 6.70% in SAVP and 2.97 to 7.05% in PSVP samples on 18^{th} day of storage.

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



Carbon dioxide favors exudation by acidifying fish muscle, so that capacity of fish proteins to hold water is reduced. Pastoriza *et al.* (1996) observed no marked effect of CO_2 on exudation of salmon slices stored in ice under CO_2 atmospheres. Resuts of the present study are in agreement with Laleye *et al.* (1984) who reported an increase in drip loss in vacuum packed pastrami during storage at 0°C and 3°C. Dalgaard *et al.* (1993) also observed an increase in drip loss during storage of vacuum packed and modified atmosphere packed cod fillets. The largest drip loss was observed for fillets stored in high CO_2 concentrations. Tiffney and Mills (1982) found that packing in 100% CO_2 increased the rate and quantity of drip loss. In all instances the amount of drip loss of fish stored in high CO_2 concentrations was higher at 0°C than at 5°C. Randell *et al.* (1997) observed that drip loss was found to be greatest in vacuum packed Baltic herring fillets compared to over-wrap packages (polystyrene or wood fibre) during storage at 2°C. Mendonca *et al.* (1989) reported that surface treatment with solutions containing (w/v) 10% potassium sorbate and 10% phosphates with or without 10% sodium acetate and 5% sodium chloride reduced exudates in vacuum packaged refrigerated pork chops compared to control samples.

Fig: 6. Changes in drip loss of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.5.2. Changes in TVB-N during chill storage

The changes in TVB-N values of Pearlspot and Black pomfret during ice storage are shown in figures 7 and 8 respectively. The TVB-N values were found to increase in all samples with storage period. The TVB-N content of Pearlspot increased from an initial value of 5.6 mg% to 19.45 in CAP and to 18.3 mg% in CVP on 10^{th} and 12^{th} 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 17^{th} day of storage. In black pomfret, the TVB-N content increased from an initial value of 4.5 mg% to 19.6, 18.4, 21.55 and 16.65 mg% in case of CAP, CVP, SAVP and PSVP samples on 10^{th}, 12^{th} and 18^{th} day of storage respectively.

TVB-N values of vacuum packed samples were lower than those of CAP samples in both the species. This is in agreement with Özogul *et al.* (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 comparatively lesser than those of control packs in both the species. Similar results have been reported by Shalini *et al.* (2001) during refrigerated storage of potassium sorbate treated vacuum packed *Lethrinus lentjan* fillets. Low levels of TVB-N in treated samples were due to either a reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Banks *et al.*, 1980). The results of the present study agree well with Rajesh *et al.* (2002) and Shalini *et al.* (2000).

TVB-N contents of potassium sorbate treated samples were slightly lower than sodium acetate treated samples in both the species. This might be attributed to the greater inhibition of H_2S producing bacteria including *S. putrefaciens* by potassium sorbate than by sodium acetate. Kim and Hearnsberger (1994) and Kim *et al.* (1995 b) 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. Botta *et al.* (1984) reported that there was a definite increase of TVB-N during ice storage of fresh Atlantic cod particularly after 9-11 days. A level of 35-40 mg% is usually regarded as limit of acceptability (Lakshmanan, 2000 and Connell, 1980). However, in the present study TVB-N values of all the samples were well within the suggested limit throughout the storage period.

Fig: 7. 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

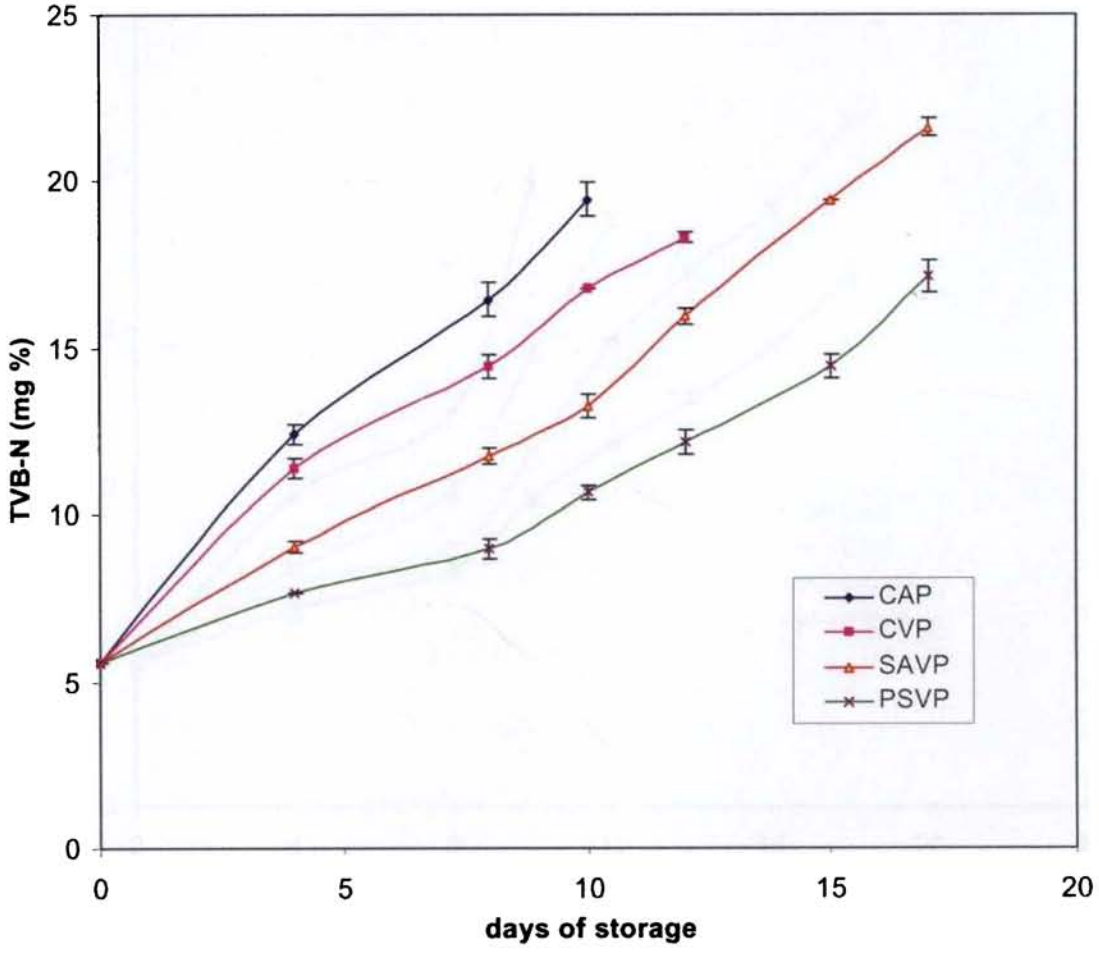
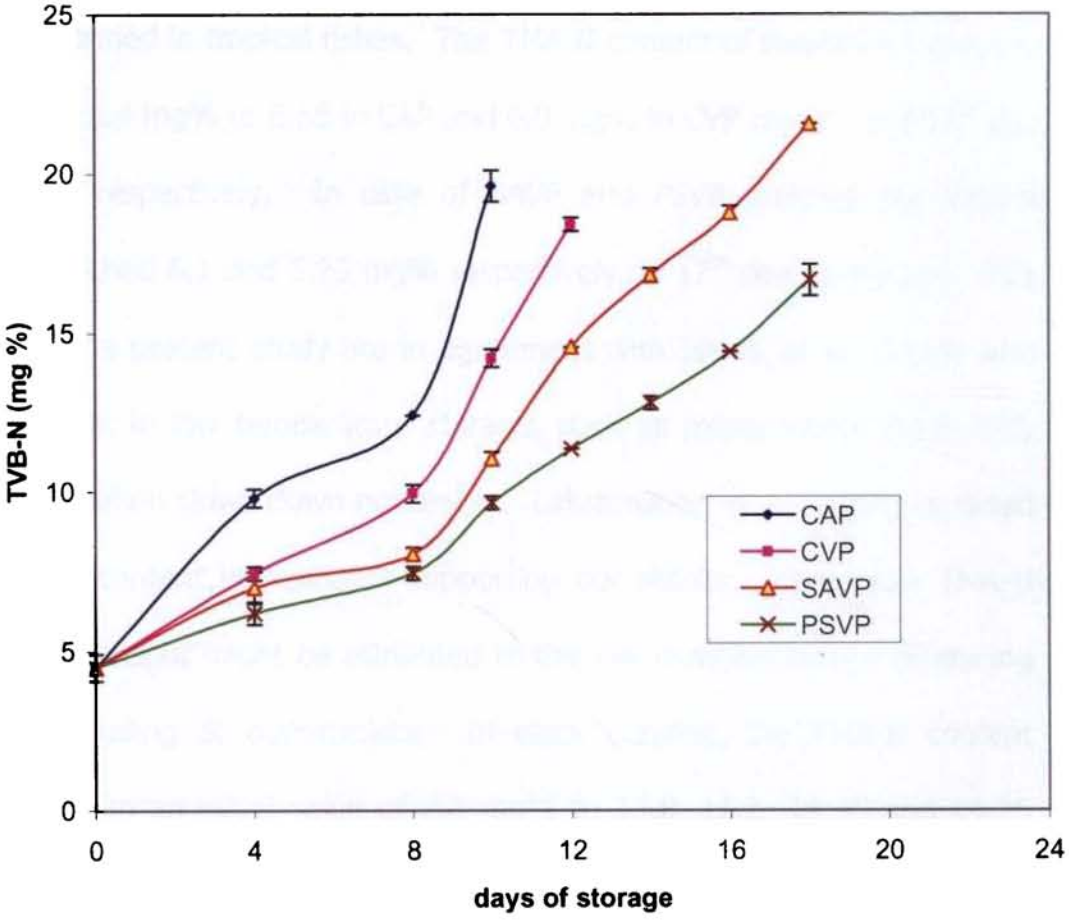


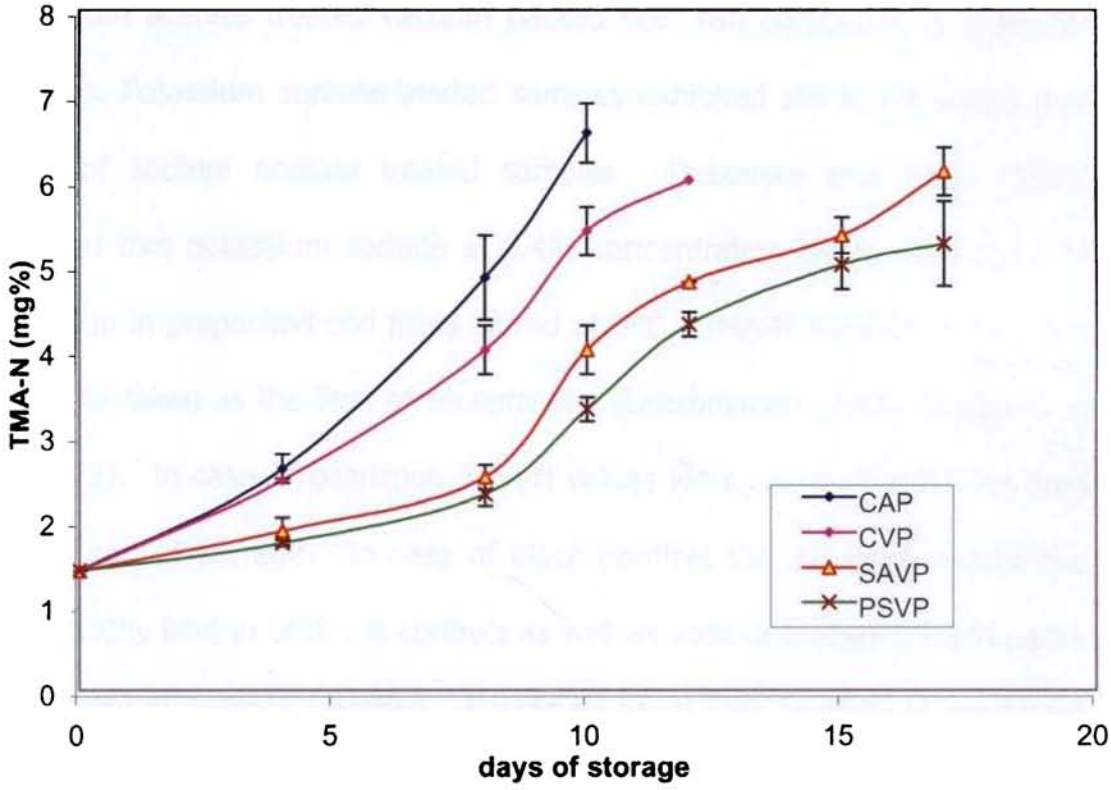
Fig: 8. Changes in TVB-N values of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.5.3. Changes in TMA-N during chill storage

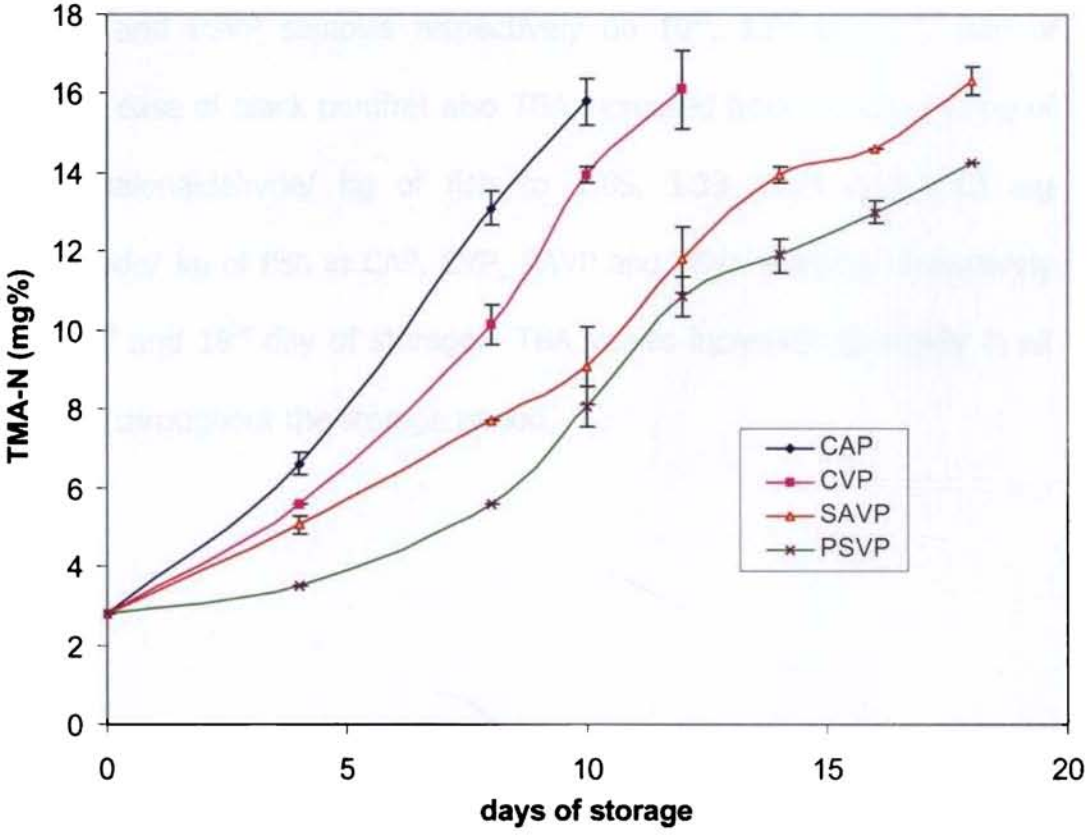
The changes n TMA-N contents of pearlspot and black pomfret during ice storage are shown in figures 9 and 10 respectively. An increasing trend was noticed in all the samples with storage time. Similar results have been reported by several authors (Parkin and Brown, 1983; Reddy *et al.*, 1995; Jensen *et al.*, 1980). Curran (1980) has demonstrated that large amounts of TMA are formed in tropical fishes. The TMA-N content of Pearlspot increased from initial 1.4 mg% to 6.55 in CAP and 6.0 mg% in CVP on 10^{th} and 12^{th} day of storage respectively. In case of SAVP and PSVP samples the TMA-N content reached 6.1 and 5.25 mg% respectively on 17^{th} day of storage. The results of the present study are in agreement with Ishida *et al.* (1976) who reported that in low temperature storage, such as refrigeration above 0°C, TMA-N formation slows down noticeably. Lakshmanan *et al.* (1996) reported low TMA-N content in Pearlspot supporting our results. These low TMA-N values in Pearlspot might be attributed to the low numbers of H_2S producing bacteria including *S. putrefaciens*. In black pomfret, the TMA-N content increased from an initial value of 2.8 mg% to 15.8, 16.1, 16.35 and 14.25 mg% in case of CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 18^{th} day of storage. The TMA-N values of air packed samples are found to be higher than those of vacuum packed samples in both the species. This is in agreement with Özogul *et al.* (2004) who observed a higher TMA values in sardine stored in air compared to that stored under vacuum.

Fig: 9. Changes in TMA-N values of Pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



The TMA-N values of treated samples are found to be comparatively lesser than those of control in both the species. This might be att ibuted to the inhibitory effect of sodium acetate and potassium sorbate over the growth of bacteria. Similar observation was made by Shalini *et al.* (2001) during refrigerated storage of potassium sorbate treated vacuum-packed *Lethrinus lentjan* fillets. Rajesh *et al.* (2002) also observed lower TMA-N values in case of sodium acetate treated vacuum packed seer fish compared to untreated samples. Potassium sorbate treated samples exhibited still lower values than those of sodium acetate treated samples. Debevere and Voets (1972) reported that potassium sorbate at 0.4% concentration (w/w) inhibited TMA formation in prepacked cod fillets stored at 0°C. TMA-N level of 10-15 mg% is usually taken as the limit of acceptability (Lakshmanan, 2000; Sengupta *et al.*, 1972). In case of pearlspot, TMA-N values were low and within the limit till the end of storage. In case of black pomfret the values exceeded the acceptability limit in both the controls as well as sodium acetate treated packs on the day of sensory rejection. A wide range of TMA level (5-26 mg/100g) has been reported for various species of spoiled fish (Castell *et al.*, 1958; Sengupta *et al.*, 1972). Parkin *et al.* (1981) have stated that TMA provides, an adequate quality index for air packaged as well as modified atmospheric packaged rock fish fillets stored at 35 ± 2°F.

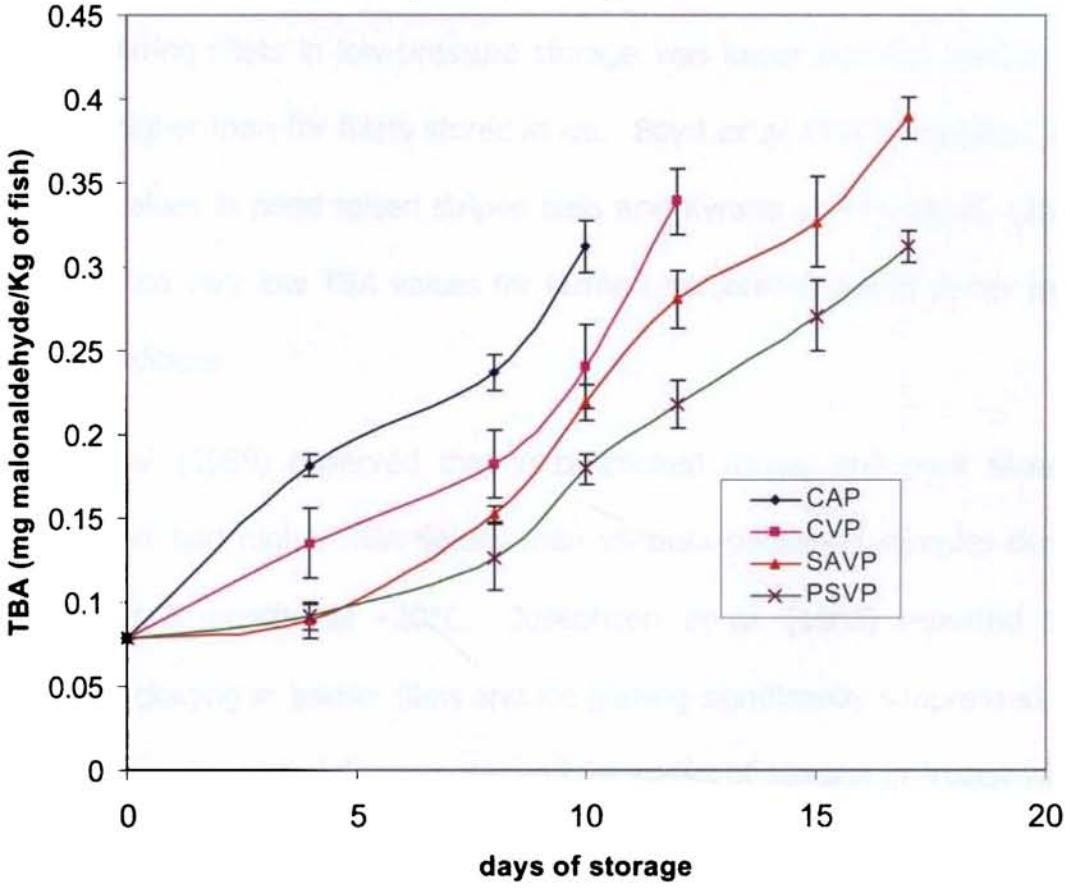
Fig: 10. Changes in TMA-N values of Black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.5.4. Changes in TBA during chill storage

The changes in TBA values of pearlspot and black pomfret during ice storage are shown in figures 11 and 12 respectively. The initial TBA value of 0.08 mg malonaldehyde/ kg of fish in case of pearlspot increased gradually during storage to 0.31, 0.34, 0.39 and 0.31 mg malonaldehyde/kg of fish in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 17^{th} day of storage. In case of black pomfret also TBA increased from an initial value of 0.28 mg malonaldehyde/ kg of fish to 1.85, 1.33, 1.14 and 1.13 mg malonaldehyde/ kg of fish in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 18^{th} day of storage. TBA values increased gradually in all the samples throughout the storage period.

Fig: 11. Changes in TBA values of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



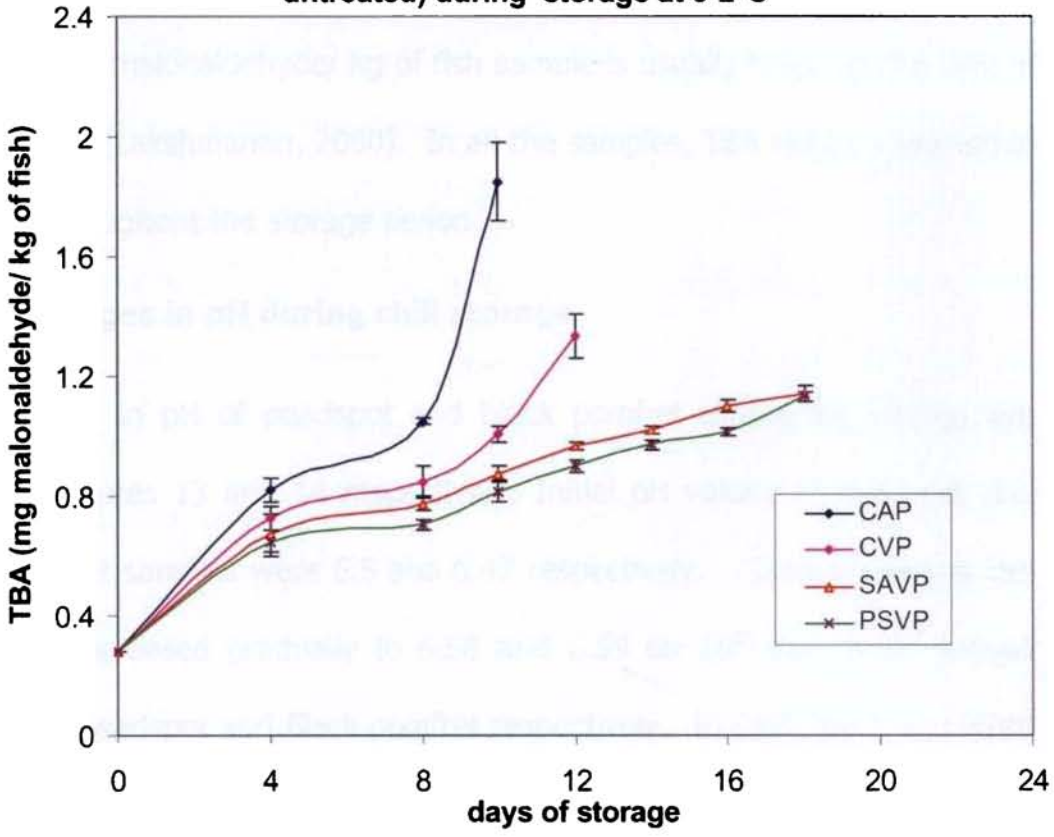
In both the species, vacuum-packed samples exhibited lower values in comparison to air packed samples. Similar observation was also made by Huang *et al.* (1994). Oxidation of fat increased during cold storage and the rate was reduced by vacuum packing and icing (Baldrati *et al.*, 1982). Varga *et al.* (1980) showed that TBA values were markedly lower in herring fillets (skin on) stored under vacuum compared to fillets stored in ice. The spoilage rate of herring fillets in low-pressure storage was lower and the storage life was 9% higher than for fillets stored in ice. Boyd *et al.* (1992) reported very low TBA values in pond raised striped bass and Kyrana and Longovis, (2002) also reported very low TBA values for farmed sea bream stored under air in chilled conditions.

Nolan *et al.* (1989) observed that in precooked turkey and pork samples stored in air had higher TBA values than vacuum packaged samples during storage for 3 months at –20°C. Josephson *et al.* (1985) reported that vacuum packaging in barrier films and ice glazing significantly suppressed the development of oxidized flavours through 24 weeks of storage in frozen white fish. Treated samples exhibited still lower values than control in both the species. This is in agreement with Rajesh *et al.* (2002) who observed a reduction in TBA values of sodium acetate treated seer fish steaks compared to control samples during chill storage.

Reduction in the amount of oxygen in modified atmospheres and the barrier characteristics of the packaging film account for the lower TBA values.

Dushyanthan *et al.* (2000) also reported a lower TBA value in case of vacuum packed beef compared to those packed under ordinary method during storage at chiller temperatures (5±2°C).

Fig: 12. Changes in TBA values of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



In case of sodium acetate and potassium sorbate treated vacuum packed samples of both the species, the values are found to be lesser than those of untreated samples. Potassium sorbate treated samples are having still lower TBA values than those of sodium acetate treated samples. TBA value in the range 1-2mg malonaldehyde/ kg of fish sample is usually taken as the limit of acceptability (Lakshmanan, 2000). In all the samples, TBA values were within the limit throughout the storage period.

4.5.5. Changes in pH during chill storage

The changes in pH of pearlspot and black pomfret during ice storage are shown in figures 13 and 14 respectively. Initial pH values of Pearlspot and Black pomfret samples were 6.5 and 6.47 respectively. During storage, the pH values increased gradually to 6.58 and 6.59 on 10^{th} day in air packed samples of pearlspot and Black pomfret respectively. In CVP, SAVP and PSVP samples of both the species, a slight decrease was noticed initially and then the values increased. Slight decrease in pH values may be attributed to the dissolution of CO_2 in the fish muscle. The extent to which pH decrease is proportional to the concentration of CO_2 in the atmosphere (Lannelongue *et al.*, 1982; Tiffney and Mills, 1982). Similar observation was made by Meekin *et al.* (1982) who reported decline in pH of vacuum packed sand flat head fillets stored at 4°C after 6 days of storage. Increase in pH may be attributed to the production of volatile base compounds by bacterial activity (Cann *et al.*, 1983). Results of the present study are in agreement with Reddy *et al.* (1995) who reported that an increase in surface pH of 100% air

packaged tilapia fillets stored at 4°C, 8°C and 16°C may be partly attributed to the production of volatile basic compounds such as ammonia by fish spoilage bacteria. Lopez Galvez *et al.* (1998) observed a pH increase in refrigerated storage of air packaged sole and only a slight increase was observed in samples stored in 20% CO_2. Dalgaard *et al.* (1993) reported that during the storage of vacuum and modified atmosphere packed cod fillets the pH increased slightly. Stammen *et al.* (1990) reported that an increase in surface pH during storage of fishery products would indicate bacterial growth and possible spoilage. The pH of potassium sorbate treated samples was lower than that of sodium acetate treated samples indicating relatively lesser production of basic compounds.

Fig: 13. Changes in pH of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

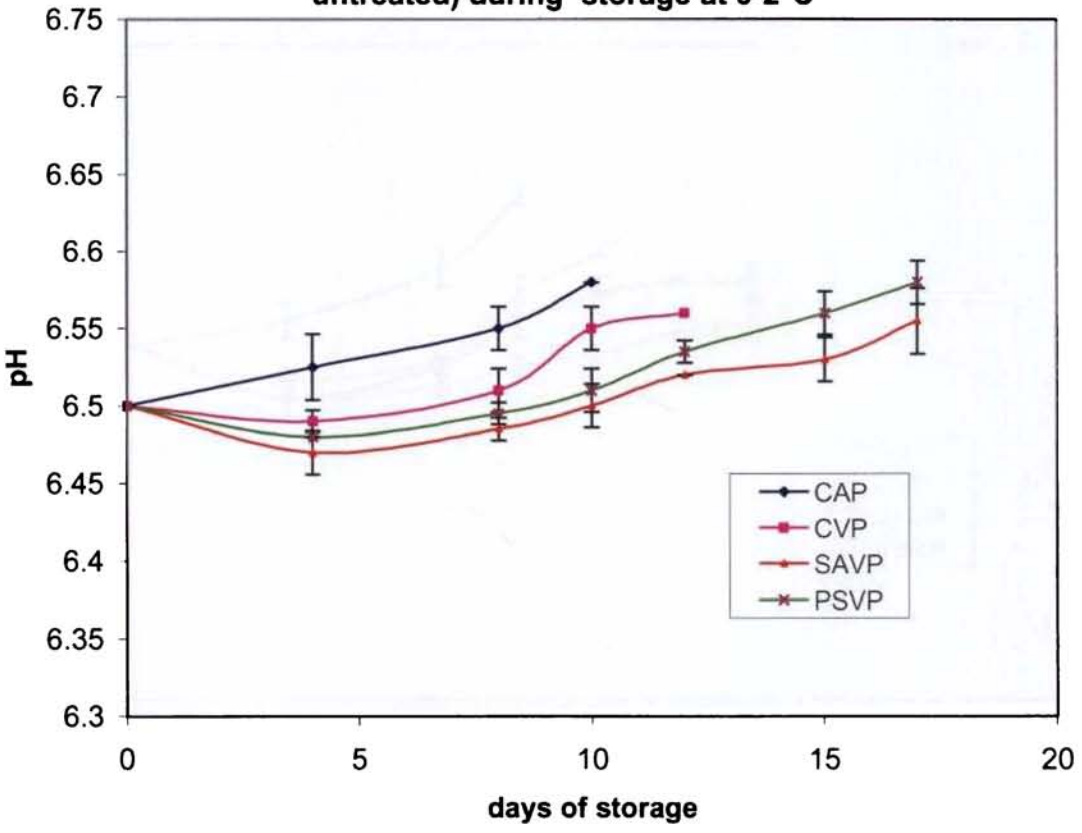
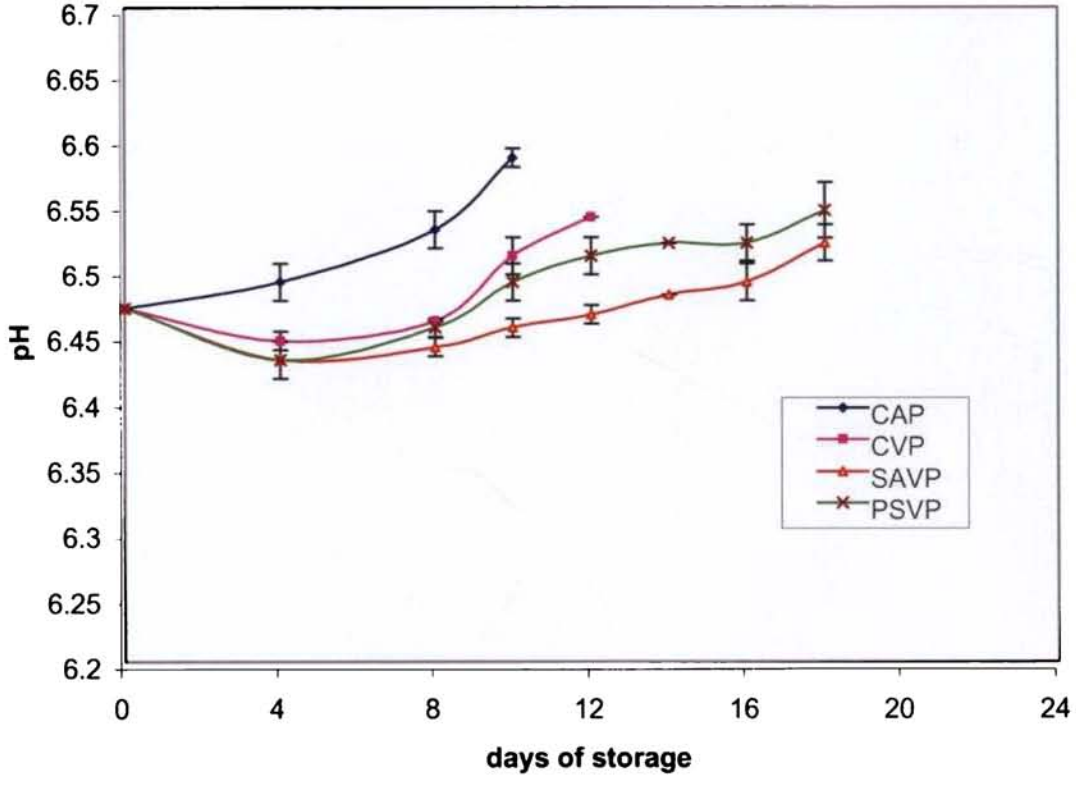


Fig: 14. Changes in pH values of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.5.6. Changes in K value

The changes in K values of pearlspot and black pomfret during ice storage are shown in figures 15 and 16 respectively. In both the species, K value was found to increase during storage. In pearlspot, K value increased from initial 4.87% to 68.7, 61.01, 68.39 and 69.4% in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 17^{th} day of storage. In black pomfret, the value increased from initial 7.81% to 67.13, 63.69, 63.43 and 62.17% in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 18^{th} day of storage.

Fig: 15. Changes in K value of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

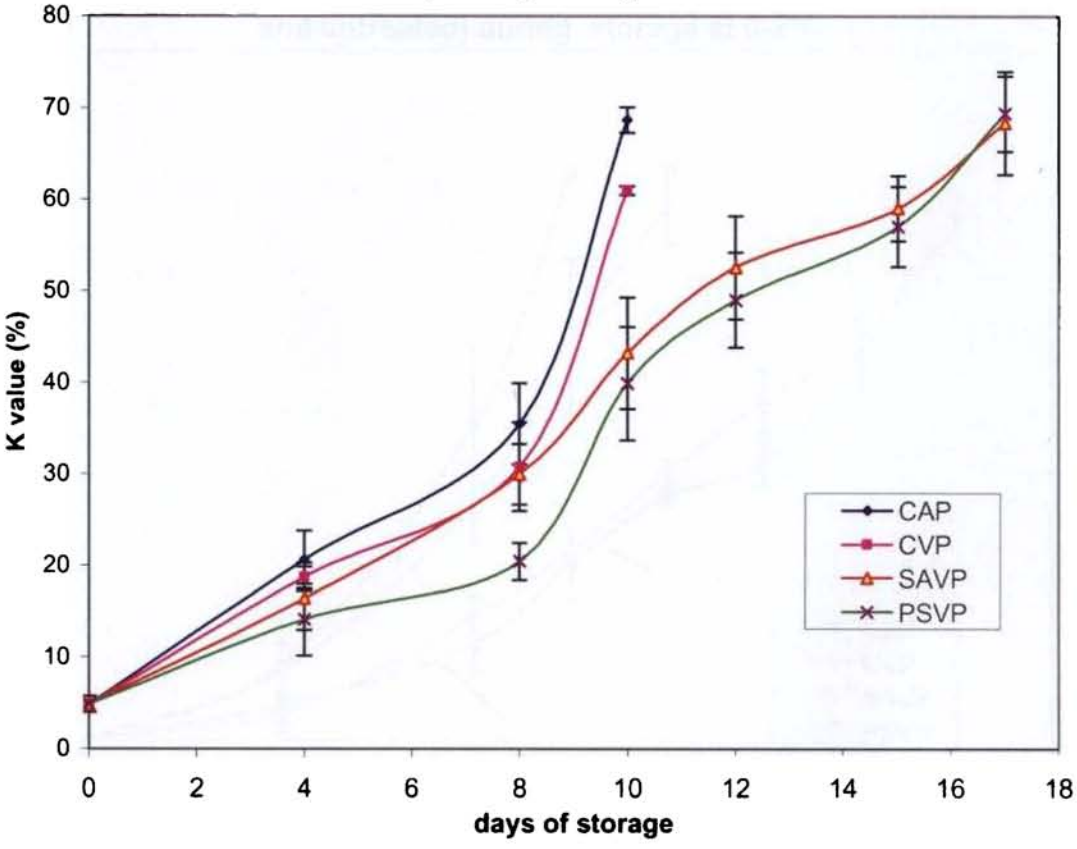
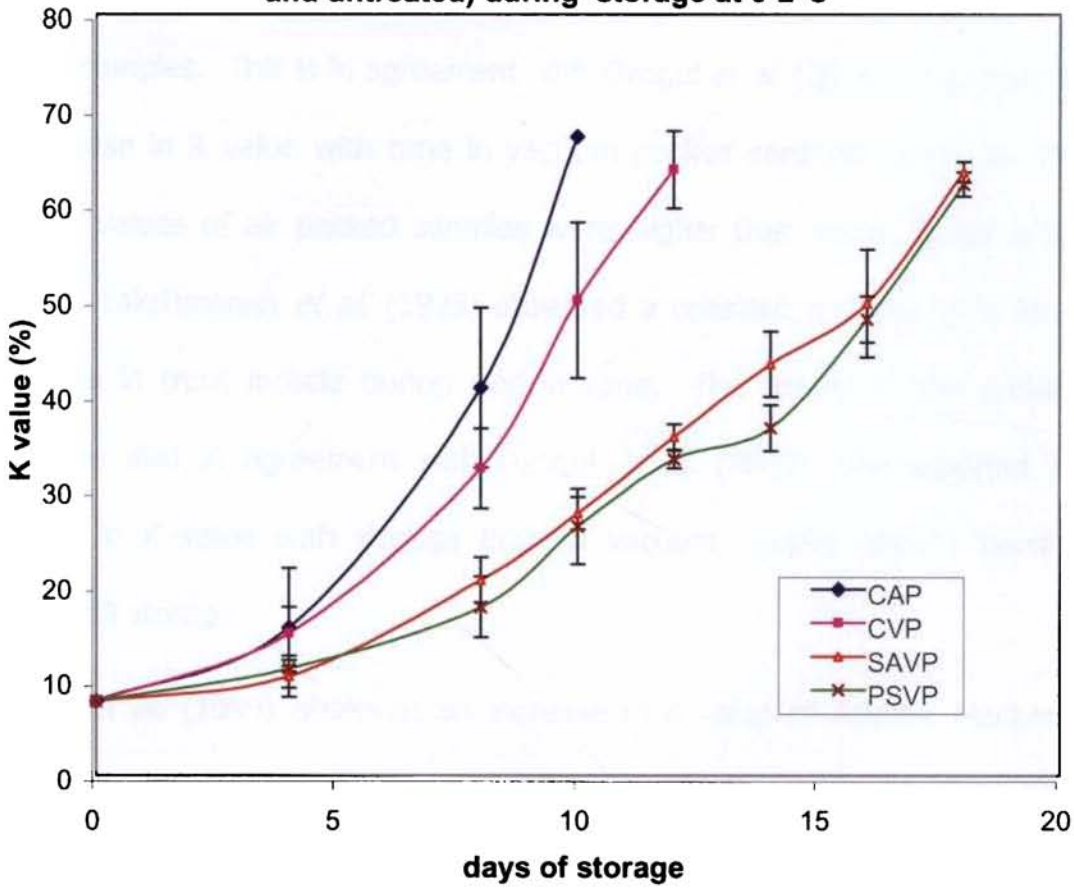


Fig: 16. Changes in K value of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



For fresh y caught fish, the initial K value reported was around 5% (Aleman *et al.*, 1982). The initial K values obtained for fresh Pearlspot and Black Pomfret in the present study is in agreement with the above observation. In both the species, an increase in 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 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 *et al.* (1993) observed a constant increase in K value with time in trout muscle during iced storage. The results of the present study are also in agreement with Özogul *et al.* (2000) who reported an increase in K value with storage time in vacuum packed Atlantic herring during chill storage.

Mathew *et al.* (1999) observed an increase in K value of Atlantic Mackerel during ice storage over 10 days. An increase in K value with time was observed by Lakshmanan *et al.* (1996) in the muscle of Pearlspot and Mullet during ice storage. The rejection levels of 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, K values of all the samples have exceeded 60%, which indicates good correlation of K value with the sensory scores.

4.6. Changes in texture

4.6.1. Changes in Hardness 1 values

The peak force during the compressive part of the test is known as hardness. It is identified as Hardness 1 for the peak during the first compression and Hardness 2 for the peak during the second compression. Changes in Hardness 1 values of pearlspot and black pomfret samples are shown in Fig. 17 and 18 respectively. In both the species, Hardness 1 values were found to decrease in control (CAP & CVP) as well as treated packs during storage. In pearlspot, hardness 1 decreased from initial 1.99 kgf to 1.86, 1.60, 1.54 and 1.47 kgf in CAP, CVP, SAVP and PSVP samples on 10^{th}, 12^{th} and 17^{th} day of storage respectively. Hardness 1 values of vacuum packed and treated samples were found to be comparatively lesser than that of air packed samples. Among SAVP and PSVP samples, the latter exhibited slightly lower hardness1 values.

In Black pomfret, hardness 1 decreased from initial 1.51 kgf to 0.92, 1.05, 0.99 and 0.86 kgf in CAP, CVP, SAVP and PSVP samples on 10^{th}, 12^{th} and 18^{th} day of storage respectively. Significant difference was not observed between hardness 1 values of treated and vacuum packed samples during storage.

Fig: 17. Changes in Hardness 1 values of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

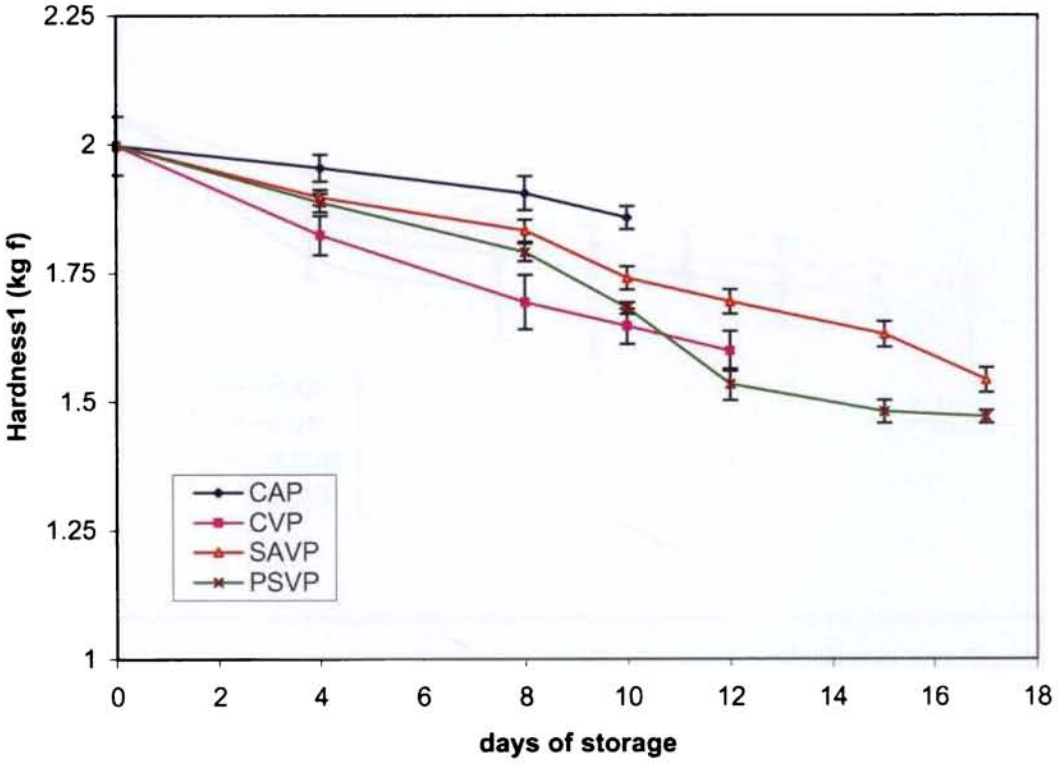
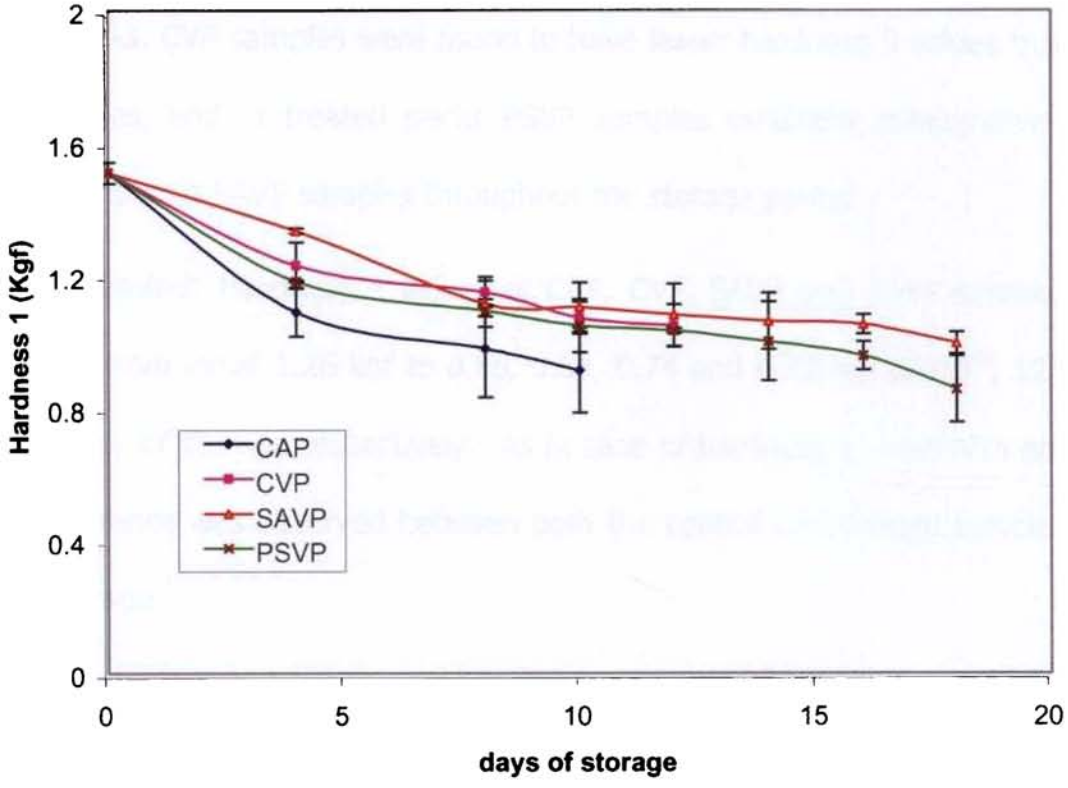


Fig: 18. Changes in hardness 1 values of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.6.2. Changes in Hardness 2 values

Changes in Hardness 2 values of pearlspot and black pomfret are depicted in figures 19 and 20 respectively. In pearlspot, Hardness 2 values of CAP, CVP, SAVP and PSVP samples decreased from initial 1.77 kgf to 1.63, 1.40, 1.32 and 1.25 kgf on 10^{th}, 12^{th} and 17^{th} day of storage respectively. In case of control packs, CVP samples were found to have lesser hardness 2 values than CAP samples, and in treated packs PSVP samples exhibited comparatively lower values than SAVP samples throughout the storage period.

In black pomfret, Hardness 2 values of CAP, CVP, SAVP and PSVP samples decreased from initial 1.26 kgf to 0.88, 0.83, 0.74 and 0.72 kgf on 10^{th}, 12^{th} and 18^{th} day of storage respectively. As in case of hardness 1, here also not much difference was observed between both the control and treated samples during storage.

Fig: 19. Changes in Hardness 2 values of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

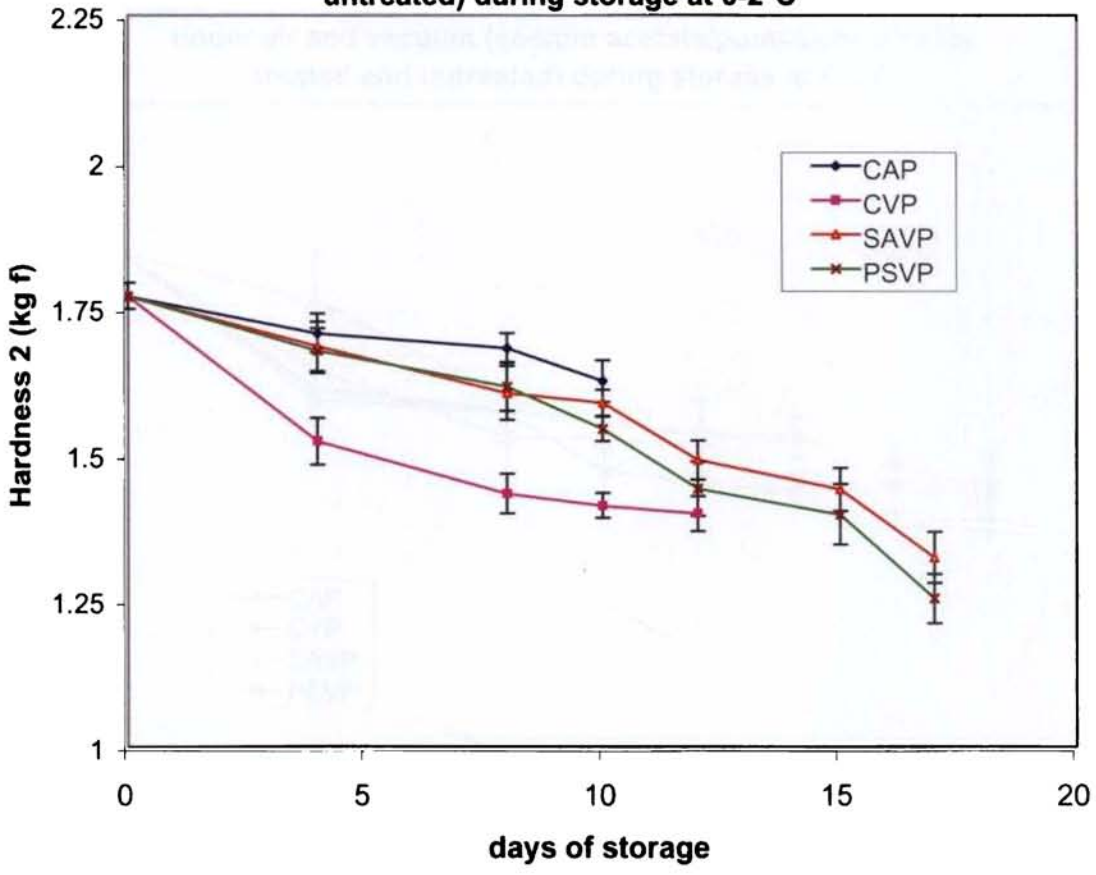
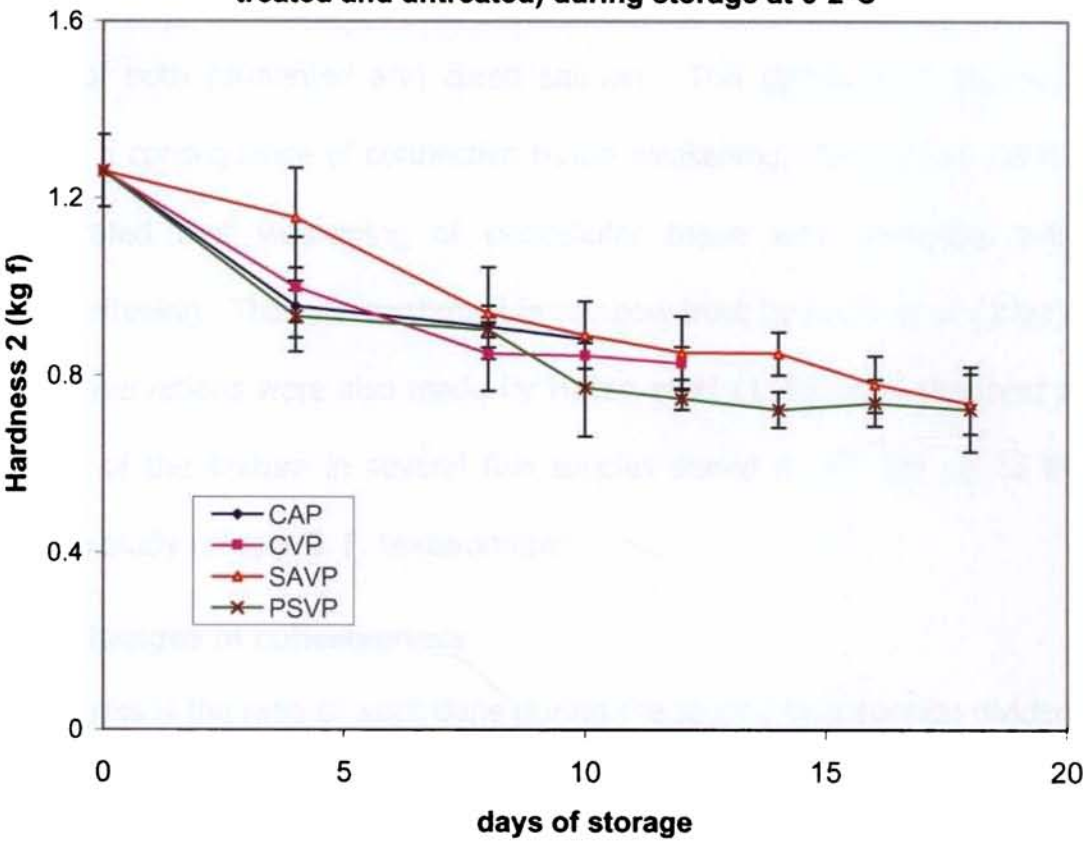


Fig: 20. Changes in Hardness 2 values of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



The results of the present study are in agreement with Azam *et al.* (1989) who studied the effect of killing method on the quality of rainbow trout during storage on ice from 0 to 15 days and observed a significant softening of both raw and cooked fillet during storage using instrumental measurement (Steven's Compression Response Analyser, SCRA). Morzel *et al.* (2000) observed that hardness (measured by TPA) decreased regularly during storage of both fermented and cured salmon. This decrease in hardness might be a consequence of connective tissue weakening. Sato *et al.* (1997) demonstrated that weakening of pericellular tissue was correlated with sardine softening. This was confirmed in rainbow trout by Ando *et al.* (1991). Similar observations were also made by Hatae *et al.* (1985) who observed a softening of the texture in several fish species stored at 4°C for up to 14 days, in a study using a G. F. texturometer.

4.6.3. Changes in cohesiveness

Cohesiveness is the ratio of work done during the second compression divided by the work done during the first compression. This result is an indication of the visco elasticity of the material. A value of 1 indicates total elasticity and a value of 0 indicates that the sample did not recover at all. Changes in cohesiveness of pearlspot and black pomfret samples during chill storage are presented in Fig. 21 and 22 respectively. In both the species values are found to decrease gradually in control as well as treated packs during storage. In pearlspot, cohesiveness decreased from an initial 0.34 to 0.28,

0.26, 0.22 and 0.22 in CAP, CVP, SAVP and PSVP samples on 10^{th}, 12^{th} and 17^{th} day of storage respectively.

In black pomfret, cohesiveness decreased from an initial 0.32 to 0.28, 0.27, 0.26 and 0.26 in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 18^{th} day of storage respectively. Significant difference was not observed in the values between control and treated samples. The values indicate that there is not much change in the internal bonding of fish muscle in both the species during storage.

Fig: 21. Changes in cohesiveness of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

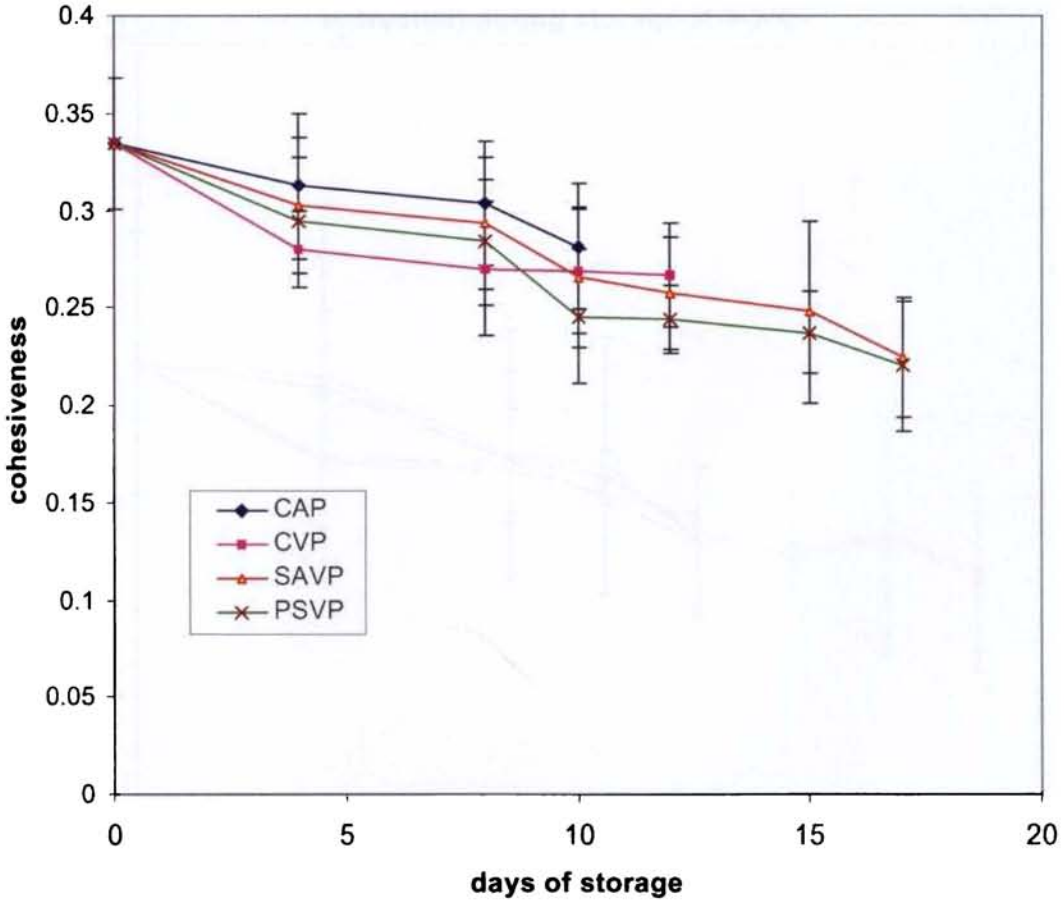
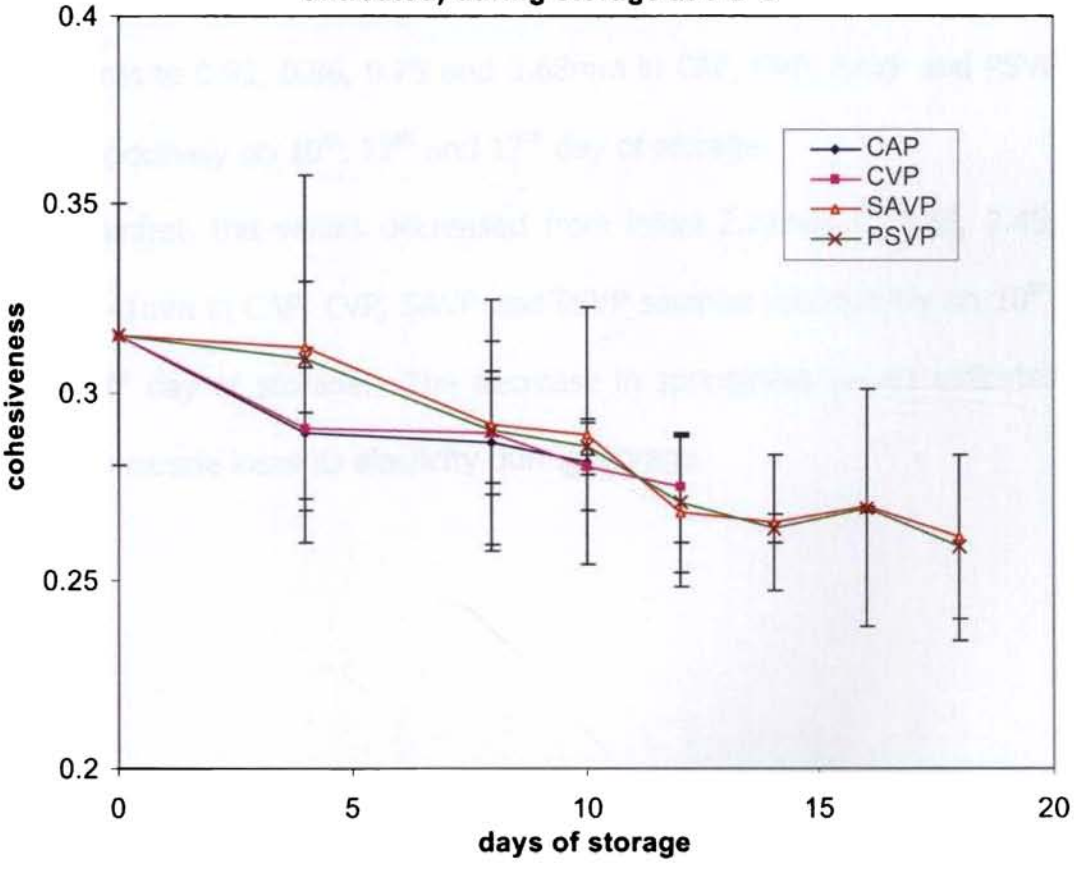


Fig: 22. Changes in cohesiveness of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.6.4. Changes in springiness

The height that the sample springs back between the end of first compression and the beginning of the second compression is known as springiness. This is sometimes referred to as elasticity. Fig. 23 and 24 represent the changes in springiness values of pearlspot and black pomfret during chill storage. In general a decreasing trend was observed in control as well as treated packs of both the species during storage. In pearlspot, springiness decreased from initial 1.01mm to 0.92, 0.86, 0.75 and 0.68mm in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 17^{th} day of storage.

In black pomfret, the values decreased from initial 2.72mm to 2.66, 2.45, 2.10 and 2.41mm in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 18^{th} day of storage. The decrease in springiness values indicates that the fish muscle loses its elasticity during storage.

Fig: 23. Changes in springiness values of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

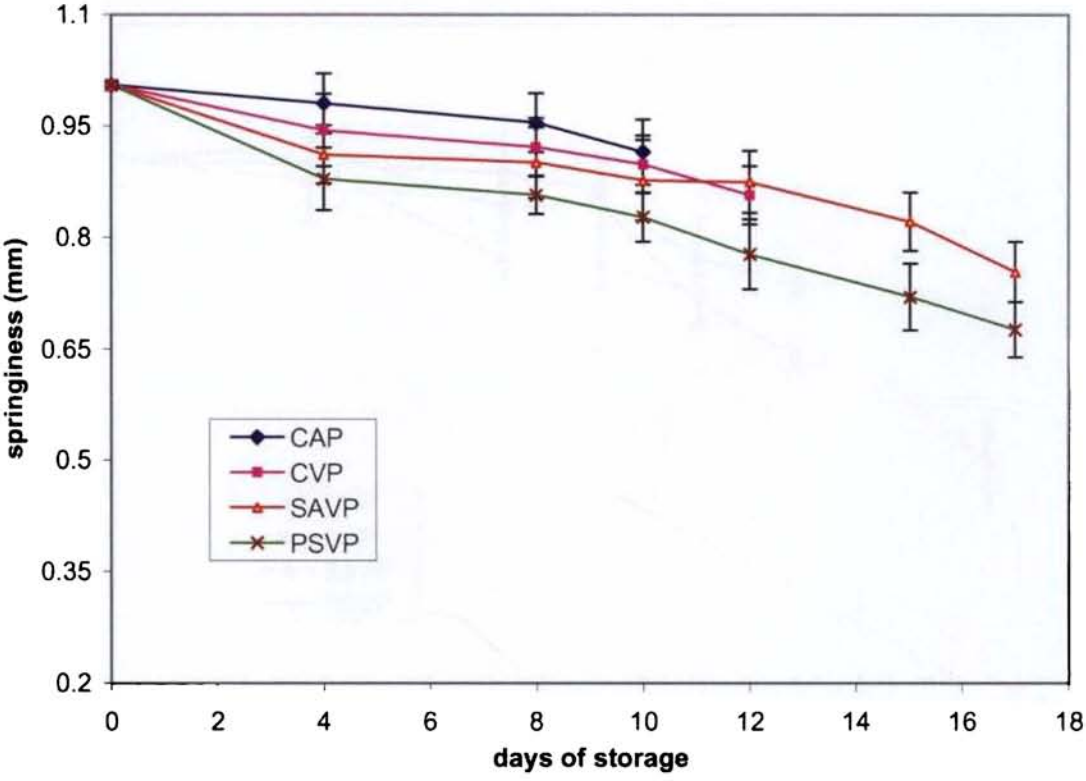
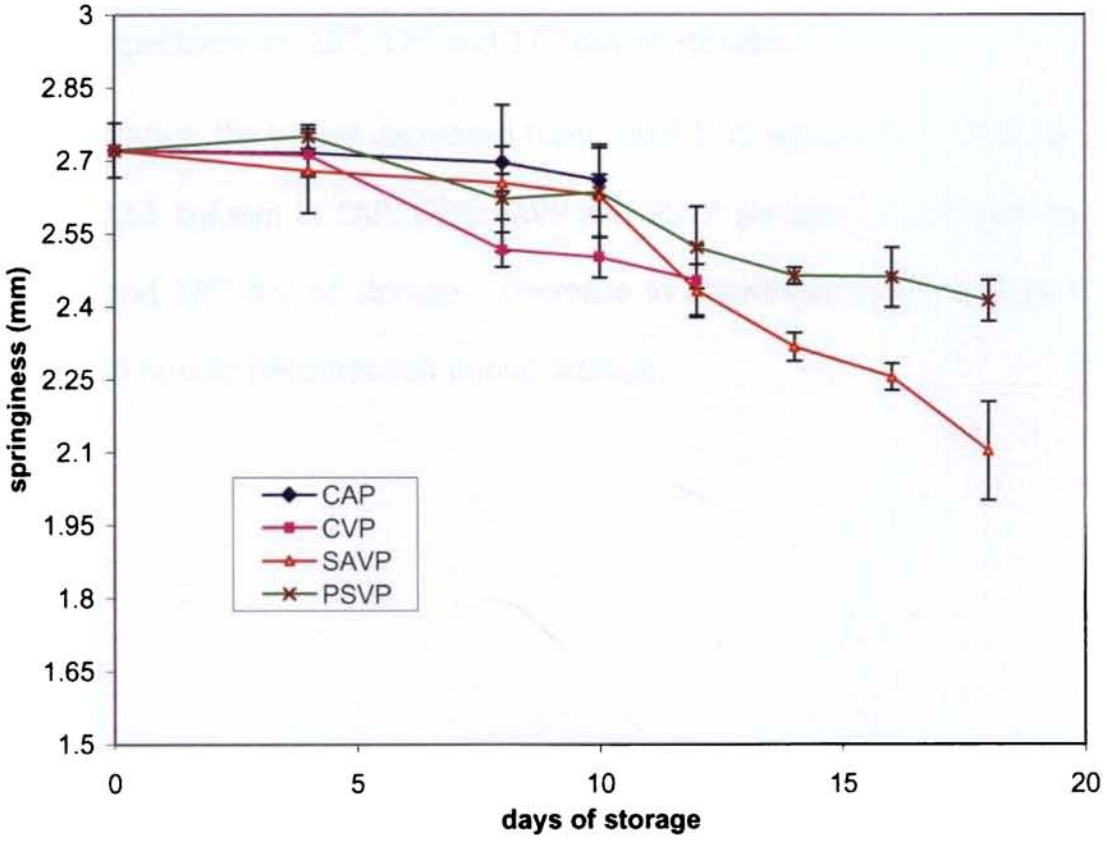


Fig: 24. Changes in springiness values of Black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.6.5. Changes in chewiness

Chewiness refers to the work done. Changes in chewiness of pearlspot and black pomfret are depicted in Fig. 25 and 26 respectively. A decreasing trend in the values was observed in control as well as treated packs of both the species during storage. In pearlspot, chewiness decreased from initial 0.65 kgf.mm to 0.48, 0.26, 0.24 and 0.23 kgf.mm in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 17^{th} day of storage.

In black pomfret, the values decreased from initial 1.35 kgf.mm to 0.85, 0.73, 0.64 and 0.53 kgf.mm in CAP, CVP, SAVP and PSVP samples respectively on 10^{th}, 12^{th} and 18^{th} day of storage. Decrease in chewiness values indicates that the fish muscle becomes soft during storage.

Fig: 25. Changes in chewiness of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

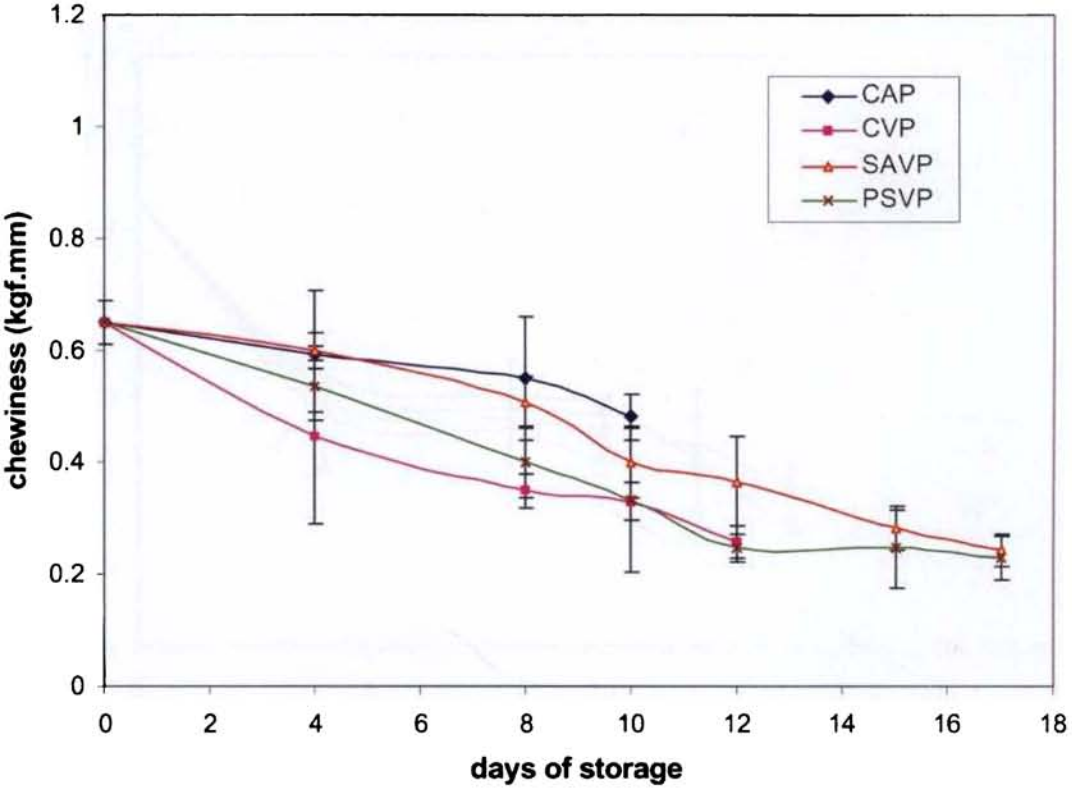
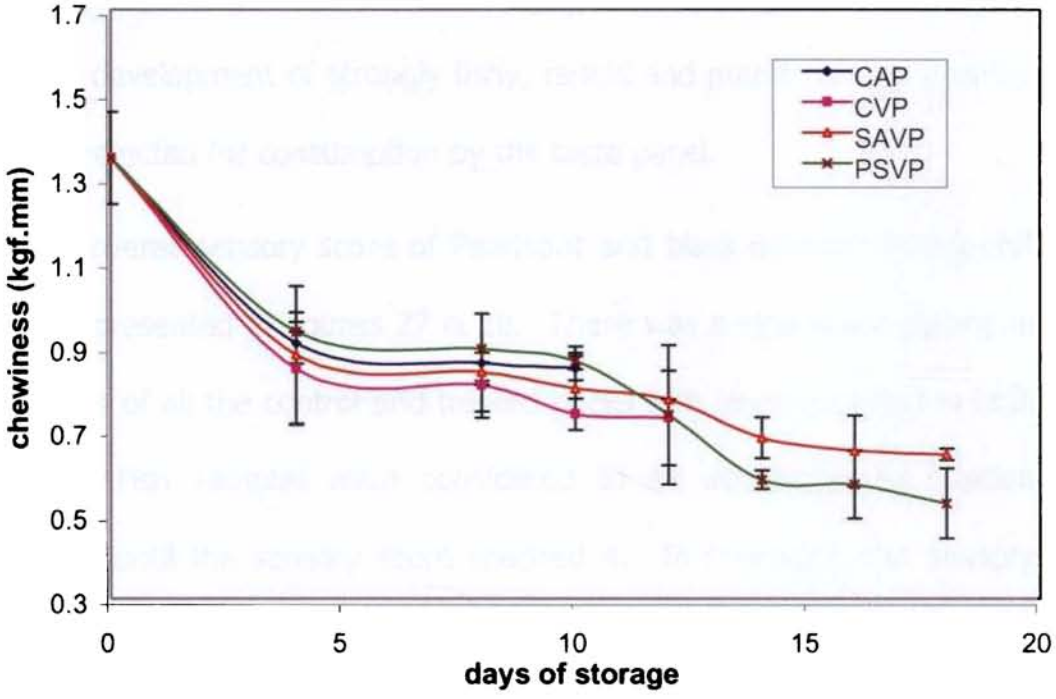


Fig: 26. Changes in chewiness of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



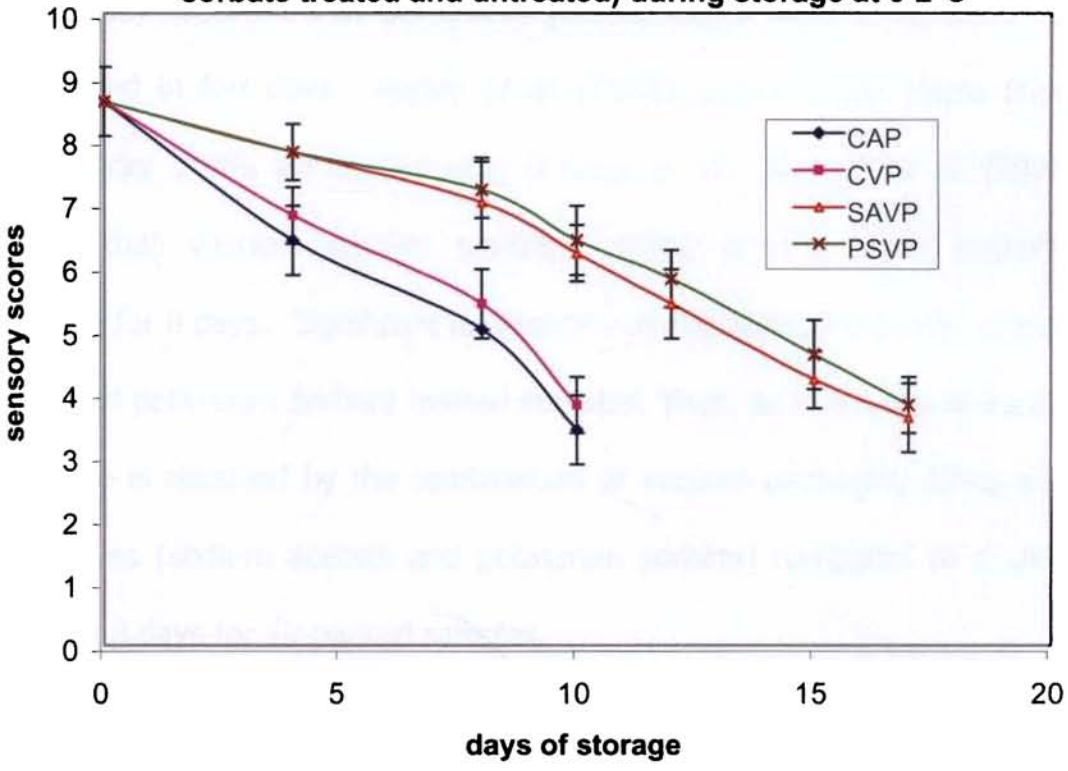
4.7. Changes in overall sensory score

The sensory characteristics finally determine whether the consumer accepts or rejects the product. Fresh pearlspot and black pomfret had characteristic brightness, sea weedy odour, with firm texture and without gaping and discoloration. Upon storage, changes were observed in sensory characteristics; texture (firm to soft), skin appearance (characteristic bright to moderately dull) and gaping (absent to slight). Fish spoilage gave rise to the subsequent development of strongly fishy, rancid and putrid odours, and fish was clearly rejected for consumption by the taste panel.

Changes in overall sensory score of Pearlspot and black pomfret during chill storage are presented in figures 27 & 28. There was a significant decline in sensory score of all the control and treated packs with storage period in both the species. 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 to 3.4 in CAP and 3.8 in CVP samples on the day of rejection. As the days of storage in ice progressed, 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. Significant difference was not noticed between sodium acetate and potassium sorbate treated samples. Thus, vacuum packaging alone didn't extend the shelf life of

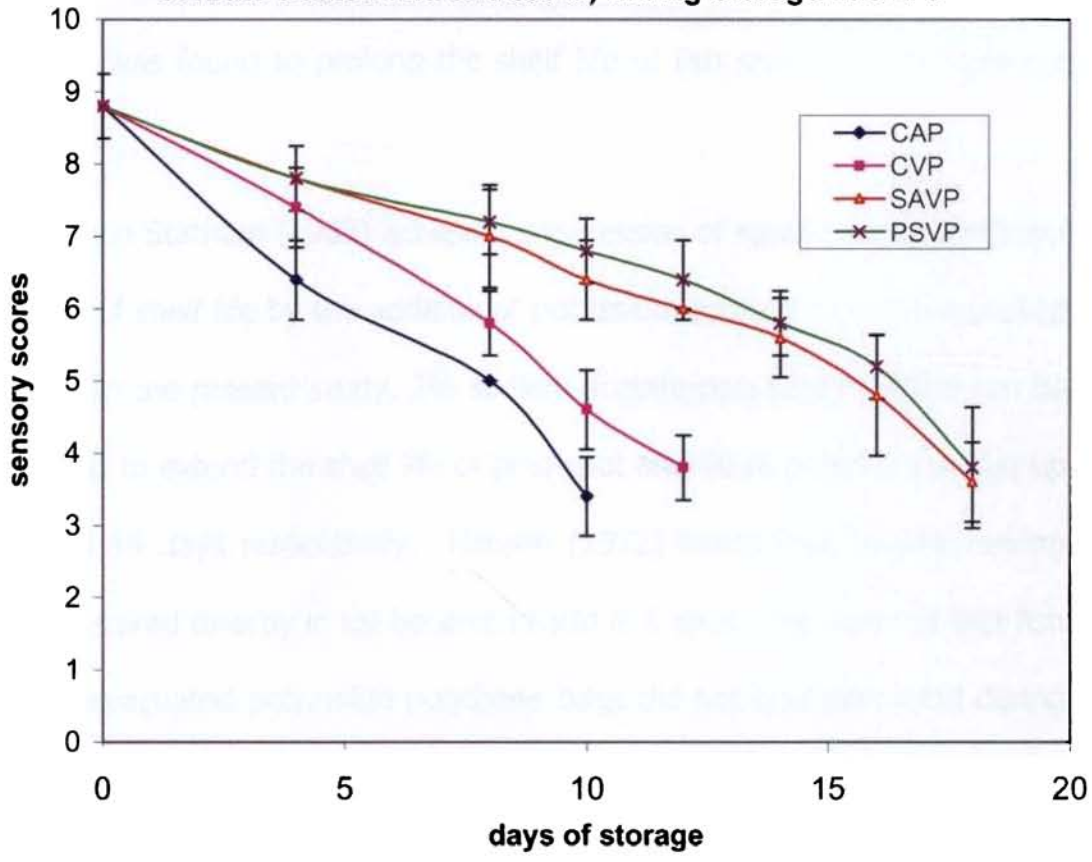
pearlspot, but vacuum packaging along with preservatives (sodium acetate / potassium sorbate) was found to extend the shelf life by 7 days compared to air packed samples.

Fig: 27. 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



In black pomfret, the sensory scores declined from an initial 8.8 to 3.4 in CAP, 3.8 in CVP, 3.6 in SAVP and 3.8 in PSVP samples on the day of rejection. 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. Significant extension was not noticed due to packing under vacuum. Meekin *et al.* (1982) reported that aerobically packed refrigerated (4°C) sand flat head spoiled in 8-9 days. Reddy *et al.* (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. Significant difference was not noticed between sodium acetate and potassium sorbate treated samples. Thus, an extension of 8 days in shelf life is obtained by the combination of vacuum packaging along with preservatives (sodium acetate and potassium sorbate) compared to a shelf life of only 8 days for air packed samples.

Fig: 28. 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



Several authors have reported shelf life for different fishes. Huss (1971) has reported 7 days shelf life for excellent quality vacuum packed cod fillets and Özogul *et al.* (2000) observed that vacuum packed herring stored at (2±2°C) were sensory acceptable for 8 days. Anon (1975) has reported 10 days shelf life for farmed trout stored at 4°C. Zhuang *et al.* (1996) has reported that the addition of sodium acetate at 2% (dip treatment) extended the shelf life of refrigerated catfish fillets. The use of sodium acetate and vacuum packaging was found to prolong the shelf life of fish samples in the present study also.

Bremner and Statham (1983) achieved suppression of spoilage and significant extension of shelf life by the addition of potassium sorbate to vacuum packed scallops. In the present study, 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. Hansen (1972) found that Atlantic herring and trout stored directly in ice became rancid in 6 days. He reported that fish stored in evacuated polyamide polythene bags did not become rancid during 20 days of storage but they developed an objectionable odour and flavour due to bacterial activity. It is emphasized that the success of vacuum packaging is completely dependent on the initial quality of the fish and adequate temperature control through out the storage (Clingman and Hooper, 1986).

4.8. Microbiological changes

4.8.1. Microbiological changes in Black pomfret stored under air and vacuum at 0-2°C

4.8.1.1. Changes in Total viable counts (TVC)

The initial total viable counts at 20°C and 37°C in black pomfret were 5.48 and 5.52 log_{10}cfu/g respectively. The changes in TVC of black pomfret samples at 20°C and at 37°C during chill storage are depicted in figures 29 and 30 respectively. Bacteria grew most quickly in black pomfret stored in air followed by those in CVP. Significant differences were observed between TVC at 37°C and 20°C at the end of storage.

10^7 cfu/g is the maximum limit for acceptability of fresh fish as recommended by International Commission on Microbiological Specification for Foods (ICMSF, 1978). In CAP samples, TVC exceeded 10^7 cfu/g on 10^{th} day when they were sensorily rejected. In CVP samples, after a lag phase of 8 days significant increase in TVC was noticed from 8^{th} day onwards and the limit count of 10^7 cfu/g was reached on 12^{th} day of storage. The lowest counts were noticed in SAVP and PSVP samples where the log phase was apparently extended. Significant difference was observed between CAP and CVP and between CAP and treated samples during the entire storage. A shelf life of 16 days was noticed for both SAVP and PSVP samples compared to 10 days for CVP and 8 days for CAP samples. The results of the study indicated that treatments with sodium acetate and potassium sorbate along with vacuum

packaging were effective in achieving an extension of shelf life of fresh Black pomfret.

Significant difference was noticed between TVC of CVP and SAVP samples after day 8. The treatment with sodium acetate significantly delayed the growth of bacteria up to 10 days. The initial mesophilic bacterial count on black pomfret is about 10^5 cfu/g in the present study. This value is within the range (10^3-10^5) established earlier for fresh tropical fish (Lima dos Santos, 1981; Gorczyka and Len, 1985 and Dalgaard *et al.*, 1993). Spoilage of most muscle foods is thought to occur when the aerobic plate count reaches 10^7 cfu/g or more (Ayers, 1960). The time required to reach 10^7 cfu/g and produce offodour was 8 days in CAP samples. The shelf life of 8 days for CAP black pomfret is comparable to that reported earlier for temperate and tropical marine fish (Lima dos Santos, 1981; Surendran *et al.*, 1989 and Özogul *et al.*, 2000). The initial total viable counts at 20°C and 37°C did not differ significantly. At the end of storage, counts at 20°C were significantly higher than that at 37°C and it indicates that considerable proportion of the bacteria easily got adapted to grow at low temperature. The shelf life of 8 days noticed in the present study may be attributed to the presence of large number of psychrotrophs and their biochemically more active nature contributing to speedier spoilage during iced storage.

It is known that a combination of low temperatures and vacuum packaging effectively reduces the growth of aerobic spoilage microorganisms (Stenstorm, 1985 and Farber, 1991). In a vacuum pack, the composition of

the gaseous phase changes during storage; the concentration of oxygen decreases while that of CO_2 increases (Gill and Molin, 1991). The bacterial flora is gradually selected towards a CO_2 tolerant but slowly growing one (Borch *et al.*, 1996). The lag phase of 8 days noticed in CVP samples in the present study can be attributed to gradual selection of CO_2 tolerant bacterial flora. Vacuum packing did not significantly extend the sensory shelf life of herring (8 days) as compared to storage in air (Özogul *et al.* 2000). In a later study Özogul *et al.* (2004) reported that TPC reached 10^7 cfu/g on day 6 in air stored sardine and on day 10 in sardine stored under vacuum pack. Shelf life of sardine based on sensory evaluation was 3 days for fish stored in air and 9 days in vacuum pack. Bacteria grew most quickly in fish stored in air at 0°C compared to vacuum pack (Leung *et al.*, 1992; Huang *et al.*, 1994; Özogul *et al.*, 2000 and Özogul *et al.*, 2004).

Prepacking treatments with organic acids and its salts effectively suppresses growth of aerobic spoilage bacteria on refrigerated fish and shrimp (Chung and Lee, 1981; Shaw *et al.*, 1983; Thakur and Patel, 1994; Kim *et al.*, 1995 b; Drosinos *et al.*, 1997; Shalini *et al.*, 2000; 2001 and Lalitha *et al.*, 2003). Significant reduction observed in TVC of SAVP and PSVP samples in the present study can be due to the preservative action of sodium acetate and potassium sorbate. Vacuum packaging with inclusion of 0.1% potassium sorbate was effective in extending the shelf life of scallops held at 4°C (Fletcher *et al.*, 1988). Gorczyka and Len (1985) reported inhibition of wide range of bacteria in potassium sorbate treated fish. Gelman *et al.* (2001)

found reduction in total viable counts in potassium sorbate treated (2.5%) pond-raised freshwater fish, Silver perch stored under air at 5°C. Extension of shelf life was reported in whole and peeled shrimp treated with potassium sorbate and bifidobacteria (Al-Dagal and Bazarra, 1999) and in catfish fillets treated with sodium acetate and bifidobacteria (Kim *et al.*, 1995 b). Brewer *et al.* (1992) reported lower population of aerobic microorganisms in beef bologna treated with 3% sodium acetate stored under vacuum at 4°C. Zhuang *et al.* (1996) showed that treatment of catfish fillets with 2% sodium acetate significantly controlled the growth of psychrotrophic bacteria. The results of the present study also confirmed the bacteriostatic nature of sorbates and acetates and their inhibitory effect on the development of the natural flora under vacuum packaging.

The results of the present study indicate that the shelf life of fresh Black pomfret depends on storage conditions (atmosphere) and on preservative treatment. Based on TVC's the shelf life of CAP samples were 8 days, CVP samples were 10 days and of SAVP and PSVP samples were 16 days.

Fig: 29. Changes in total viable counts (20°C) of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

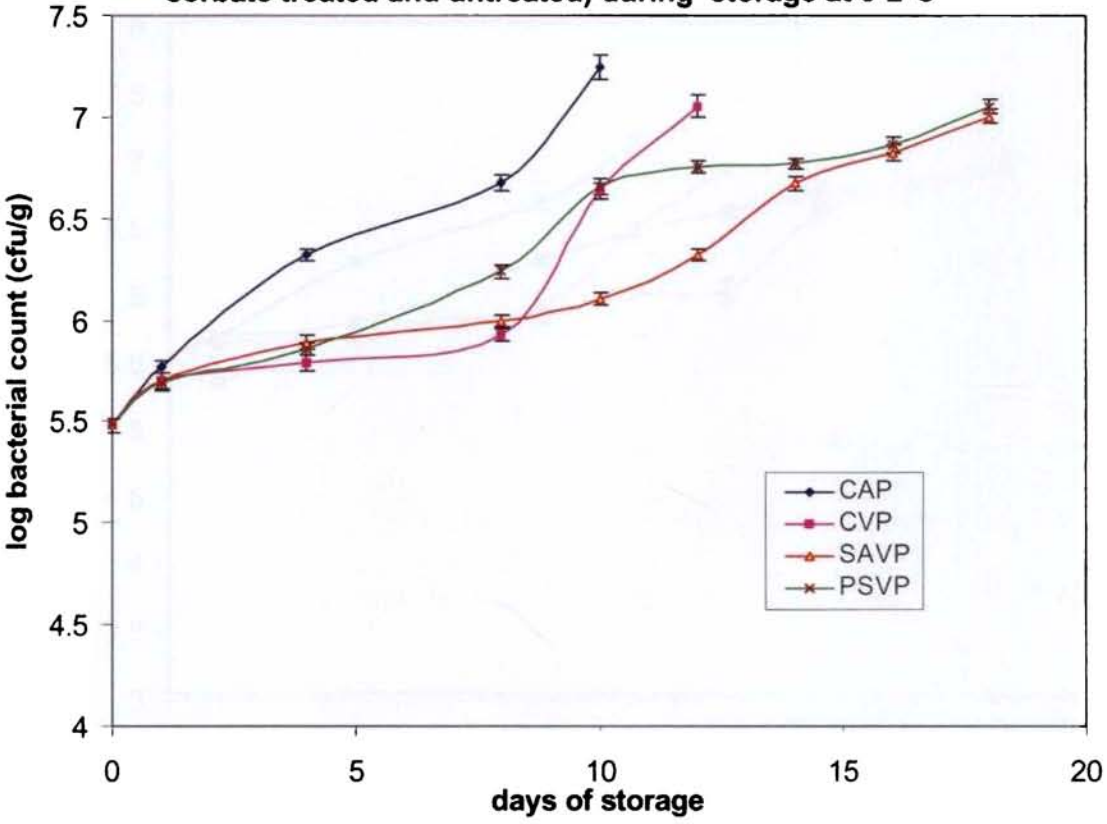
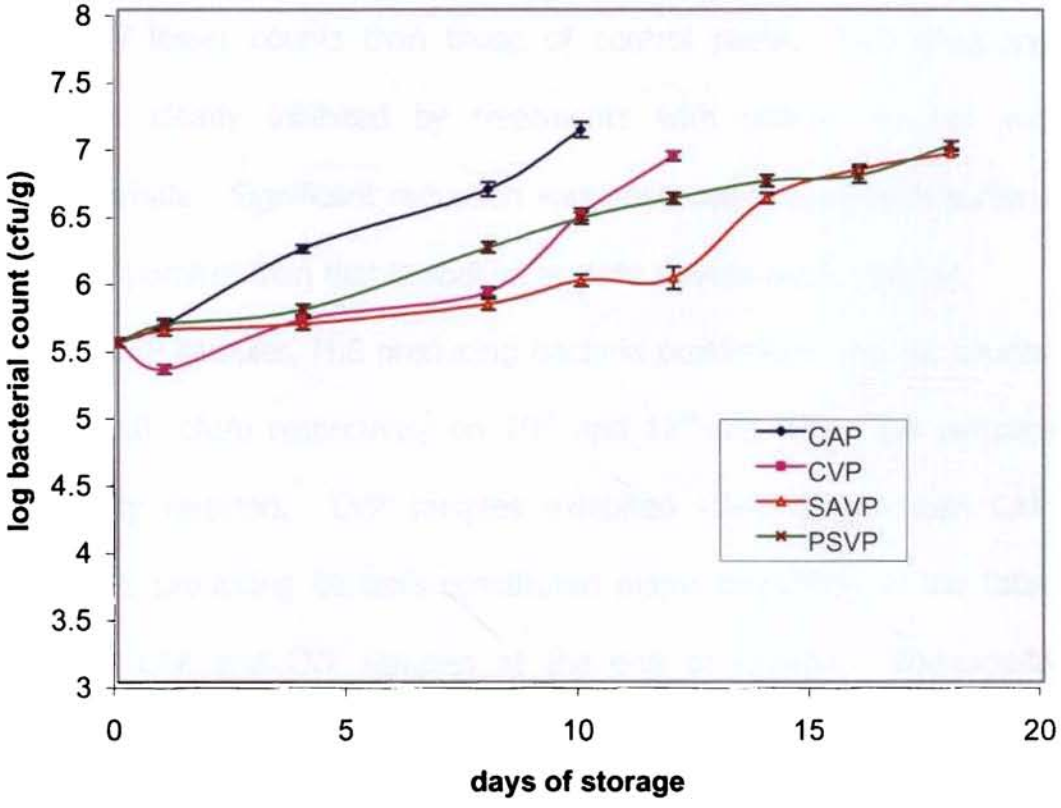


Fig: 30. Changes in total viable counts (37°C) of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.8.1.2. Changes in H_2S Producing bacterial count

The initial concentration of H_2S producing bacteria in Black pomfret was 10^{3}-10^{4} cfu/g and it constituted 1% of the total viable counts at day 0. H_2S producing bacteria presented very high growth between 0 day and 4th day in all the samples, possibly due to increased substrate temperature (Fig: 31). After 4 days of storage in ice, treated samples, SAVP and PSVP showed comparatively lesser counts than those of control packs. H_2S producing bacteria was clearly inhibited by treatments with sodium acetate and potassium sorbate. Significant reduction was observed in potassium sorbate treated black pomfret than that in sodium acetate treated black pomfret.

In CAP and CVP samples, H_2S producing bacteria proliferated and the counts reached ca. 10^{7} cfu/g respectively on 10th and 12th day when the samples were sensorily rejected. CVP samples exhibited lower counts than CAP samples. H_2S producing bacteria constituted major proportion of the total microflora in CAP and CVP samples at the end of storage. *Shewanella putrefaciens* dominated the H_2S producing flora in CAP and CVP. The results of the present study agree well with that of Lyhs *et al.* (2001) and Goulas *et al.* (2005). Lyhs *et al.* (2001) observed that H_2S producing bacteria constituted a higher proportion of bacterial flora during storage of vacuum packaged 'gravad' rainbow trout slices at 3°C and 8°C. They also showed that CVP samples exhibited comparatively lower counts than CAP samples. Goulas *et al.* (2005) reported that vacuum packaging delayed the growth of H_2S producing bacteria compared to air packed samples during storage of

aqua cultured mussels at 4°C. The H_2S producing bacterial counts reached 5.26 and 4.65 log_{10}cfu/g in SAVP and PSVP samples of black pomfret respectively when they were rejected based on sensory scores. Proliferation of H_2S producing bacteria was prevented by treatment with potassium sorbate.

Shewanella putrefaciens is a well known fish spoilage bacteria that produce H_2S, intensive off-odours and TMA, and this organism was found responsible for spoilage of vacuum packed cod (Dalgaard, 1995). In the present study, black colonies isolated from Iron Agar plates were identified as *S. putrefaciens*. The highest counts of H_2S producing bacteria including *S. putrefaciens* were found in CAP samples where they had reached 10^7 cfu/g on 10^{th} day.

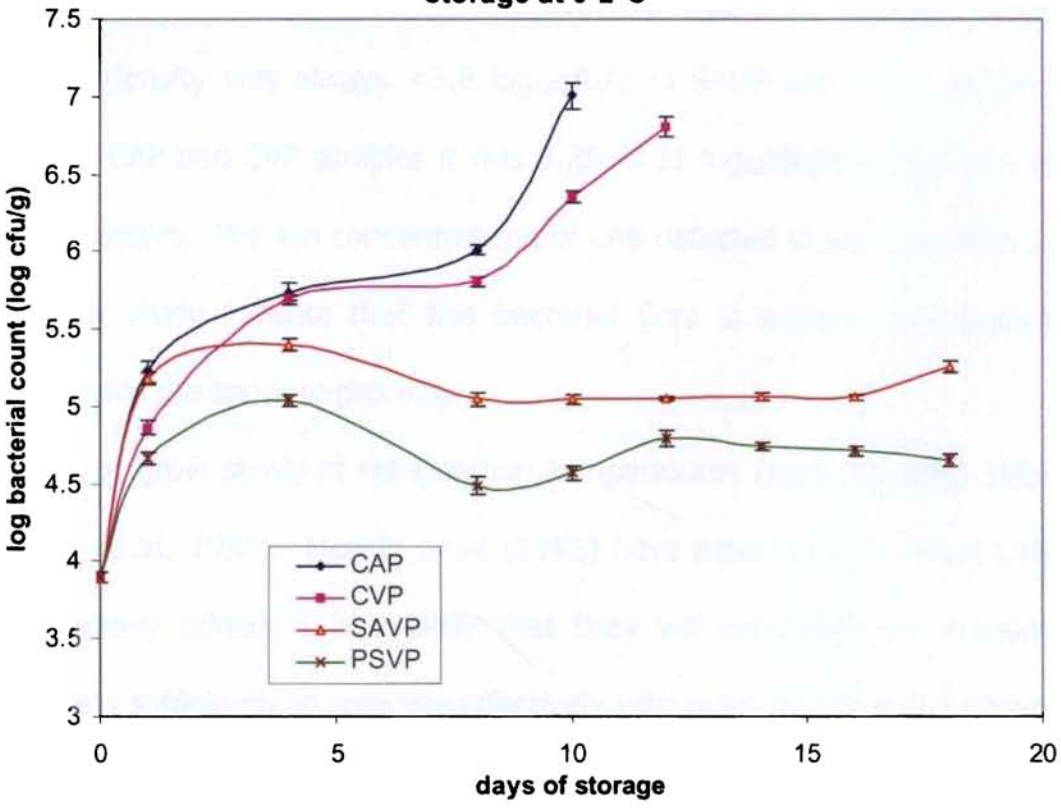
The initial level of H_2S producing bacteria (3.8 log_{10}cfu/g) reported in the present study is comparable to that of Mediterranean Bouque (Koutsoumanis and Nychas, 1999) and gilt head seabream (Drosinos *et al.*, 1997). Dalgaard (1995) also found H_2S producing bacteria at levels of 10^5-10^7 cfu/g in spoiled vacuum packed cod. Gram *et al.* (1987) identified *S. putrefaciens* as the principal spoiler of vacuum packed cod fillets and at rejection time, the number of H_2S producing organism varied from 10^6-10^7 cfu/g. Jorgensen and Huss (1989) found *S. putrefaciens* was responsible for spoilage of VP cod fillets and suggested that the low number of bacteria found at rejection 10^5-10^6 cfu/g was caused by variation in spoilage potential amongst strains of H_2S producing organisms. It was suggested that CO_2 inhibited growth of H_2S

producing bacteria in vacuum packs (Jorgensen *et al.*, 1988). Gram *et al.* (1987) reported that the number of H_2S producing bacteria at rejection constituted a large percentage, i.e. 70-80% of the total count in vacuum packed cod fillets at 0°C compared to 40-50% for whole fish. El Marrakchi *et al.* (1990) reported that H_2S producing flora represented only 1% of the total microflora present at day 0, its proportion reached 50% when the fish was clearly spoiled. The results of the present study agree well with that of Gram *et al.* (1987) and El Marrakchi *et al.* (1990). The number of H_2S producing bacteria at rejection of vacuum packed samples constituted 60-70% of the total count while that of CAP was 30-40%. *S. putrefaciens* was identified as the main spoilage organism of black pomfret stored under air and vacuum at 0-2°C as reported earlier for haddock fillets (Chai *et al.*, 1968) and cod (Gram *et al.*, 1987).

In the present study, significant reduction in H_2S producing bacterial counts were noticed in SAVP and PSVP samples compared to that of CAP and CVP samples. Inhibition of *S. putrefaciens* by sorbate in fresh fish stored under aerobic condition has been reported earlier (Thakur and Patel, 1994). It has been reported that the conjunction of vacuum or MAP can affect the growth/survival of H_2S producing bacteria including *S. putrefaciens* in fish treated with sorbate. Drosinos *et al.* (1997) reported reductions in H_2S producing bacteria in gilt head seabream fillets treated with potassium sorbate and stored under MAP at 0°C.

Sodium acetate was effective in suppressing growth of H_2S producing bacteria in vacuum packed black pomfret. However, among the two treatments, sorbate treatment was found to be better than acetate treatment for preventing the proliferation of H_2S producing bacteria. Kim and Hearnsberger (1994) reported that sodium acetate was effective in suppressing growth of aerobic spoilage bacteria on refrigerated catfish fillets. In another study Kim *et al.* (1995 b) found that sodium acetate, either alone or in combination with bifidobacteria was effective in suppressing aerobic spoilage bacteria and extended refrigerated shelf life of catfish fillets. Treatments with sodium acetate and potassium sorbate suppressed the growth of H_2S producing bacteria by extending the log phase and thereby extending shelf life.

Fig: 31. Changes in counts of H_2S producing bacteria of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.8.1.3. Changes in Lactic acid bacteria (LAB)

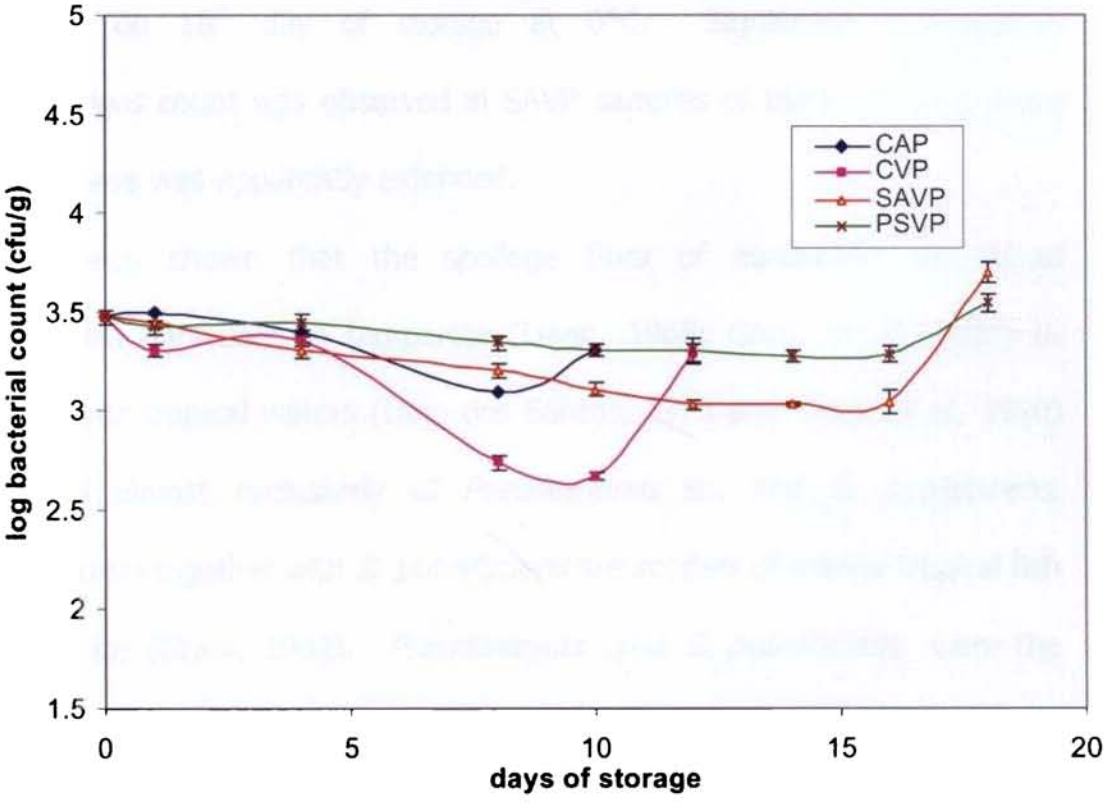
The initial level of Lactic acid bacteria in black pomfret was 3.47 log_{10}cfu/g. The changes in LAB in black pomfret samples stored under air and vacuum with and without treatments are shown in fig. 32. The LAB count decreased or remained almost constant in CAP and CVP samples. In SAVP and PSVP samples, their count decreased in the first two weeks of storage. Their population density was always <3.8 log_{10}cfu/g in SAVP and PSVP samples whereas in CAP and CVP samples it was 3.29 -3.31 log_{10}cfu/g at the time of sensory rejection. The low concentrations of LAB detected in black pomfret in the present study indicate that this bacterial flora is without quantitative importance for the spoilage process.

LAB tends to grow slowly at refrigeration temperatures (Huis in't Veld, 1996 and Gopal *et al.*, 1996). Meekin *et al.* (1982) have reported that unless LAB are deliberately added, it is unlikely that they will proliferate on vacuum packed fillets sufficiently to compete effectively with gram negative facultative anaerobes. Bremner and Statham (1983) reported that *Lactobacillus* was not present after 13 days of storage in vacuum packed scallops. Lyhs *et al.* (2001) also observed lower LAB counts than other determined bacterial counts in vacuum packed 'gravad' rainbow trout slices during storage at 3°C. In contrast, Pedersen and Snabe (1995) reported that spoilage of vacuum packed tropical fish is caused by lactic acid bacteria. Under anaerobic conditions at temperature below 20°C, psychrotrophic LAB, which are capable

of growth at 5°C or below, enable successful competition with other psychrotrophic spoilage microorganisms (Doyle *et al.*, 1997).

Potassium sorbate was effective in delaying the growth of lactobacilli in vacuum packaged pork chops (Mendonca *et al.*, 1989). Unda *et al.* (1990) reported that lactobacilli populations in vacuum-packaged beef steaks multiplied only after 2 weeks at 2-4°C and in potassium sorbate, phosphate, sodium chloride and sodium acetate treated beef steaks, inhibition of lactobacilli was noticed. Kemp *et al.* (1989) reported low lactobacilli counts (1 log_{10}cfu/g) in vacuum packaged dry cured ham slices stored at 2°C at week 6 and thereafter gradually increased and reached 4.0 log_{10}cfu/g at 8 weeks. Shalini *et al.* (2000) reported increase in LAB during the first two weeks of refrigerated storage of *Lethrinus lentjan* fillets treated with sodium acetate (1.0%, 1.5% and 2%) and thereafter no growth was noticed and they attributed this to suppression of growth by other nutritionally competitive organisms. The results of the present study indicate that sodium acetete and potassium sorbate are effective in delaying the growth of LAB during the first two weeks of storage of black pomfret at 0-2°C. LAB is not likely to be responsible for spoilage of CVP, SAVP and PSVP samples of black pomfret.

Fig: 32. Changes in Lactic Acid Bacterial counts of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.8.1.4. Changes in counts of *Pseudomonas*

The changes in counts of *Pseudomonas* in black pomfret samples during chill storage are depicted in fig. 33. Significant increase in their numbers was noticed after one week storage in CAP, CVP and PSVP samples of black pomfret. In CAP and CVP samples, *Pseudomonas* count reached 5.1 log_{10}cfu/g and 4.45 log_{10}cfu/g respectively when they were sensorily rejected. In PSVP samples, population density gradually increased and reached 4.23 log_{10}cfu/g on 18^{th} day of storage at 0°C. Significant reduction in *Pseudomonas* count was observed in SAVP samples of black pomfret where the log phase was apparently extended.

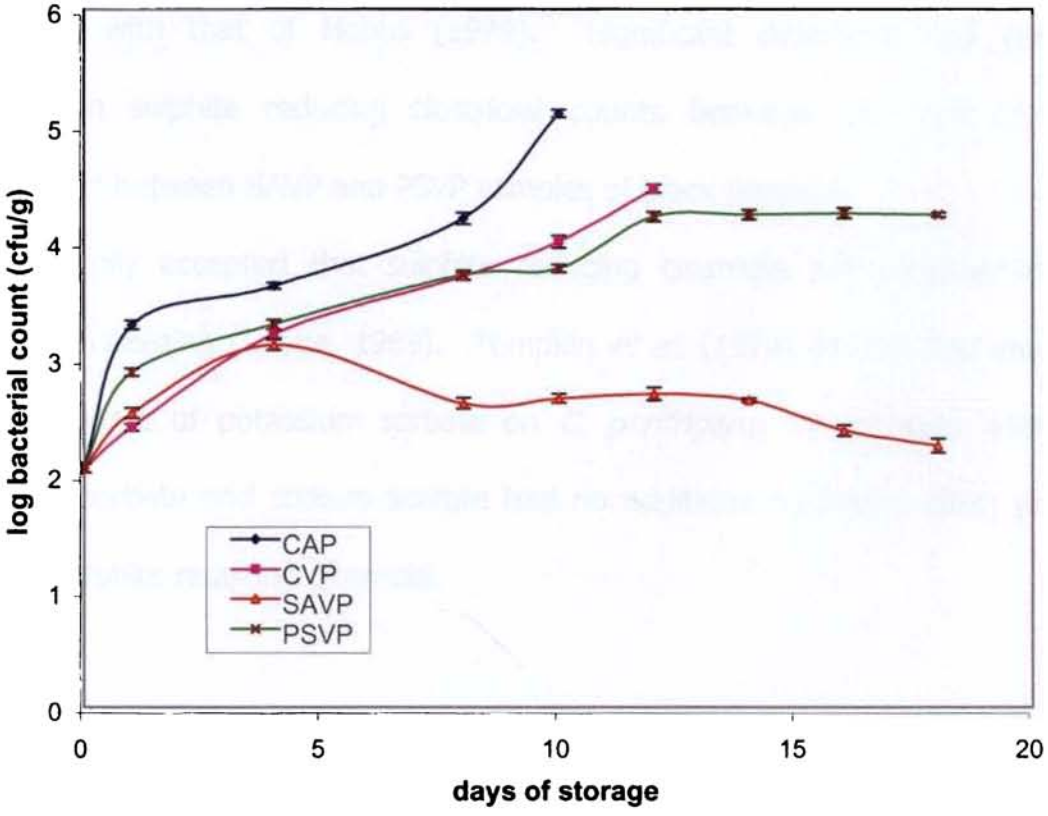
It has been shown that the spoilage flora of aerobically ice stored fish/shellfish harvested in temperate (Levin, 1968; Gram *et al.*, 1987) or subtropicular tropical waters (Lima dos Santos, 1978 and Gram *et al.*, 1990) composed almost exclusively of *Pseudomonas* sp. and *S. putrefaciens*. *Pseudomonas* together with *S. putrefaciens* are spoilers of marine tropical fish stored in ice (Gram, 1992). *Pseudomonas* and *S. putrefaciens* were the dominant bacteria at the end of storage of Mediterranean Bouque stored aerobically at 0°C (Koutsoumanis and Nychas, 1999). The results of the present study agree well with that of Gram (1992) and Koutsoumanis and Nychas (1999). In contrast to this Surendran *et al.* (1989) reported that 75-81% of the flora of spoiled tropical marine fish consisted of *Pseudomonas* alone.

Several investigators have reported that in vacuum packed ice stored fish from temperate marine waters, the number of *pseudomonas* is reduced but *S. putrefaciens* grows to levels of 10^6-10^8 cfu/g (Gram *et al.*, 1987; Jorgensen *et al.*, 1988; Dalgaard *et al.*, 1993). Jorgensen *et al.* (1988) and Dalgaard *et al.* (1993) reported that the spoilage of vacuum packed fish from temperate marine waters is caused by *S. putrefaciens* and *Photobacterium phosphoreum.* Goulas *et al.* (2005) observed a delay in the growth of *Pseudomonas spp.* in vacuum packed aqua cultured mussels compared to air packed samples during storage at 4°C. The results of the present study on CVP samples agree well with that of Gram *et al.* (1987), Jorgensen *et al.* (1988), Dalgaard *et al.* (1993) and Goulas *et al.* (2005) in that *S. putrefaciens* grew to levels of ca. 10^7cfu/g and the numbers of *Pseudomonas* were reduced. In contrast to this, Pedersen and Snabe (1995) reported that spoilage of vacuum packed tropical fish stored in ice is caused by lactic acid bacteria.

Treatments with potassium sorbate and sodium acetate were effective in controlling growth of *Pseudomonads* in fish packed under air and MAP (Kim *et al.*, 1995 b and Drosinos *et al.*, 1997). Significant reductions were noticed in *Pseudomonas* count of gilt-head seabream fillets treated with potassium sorbate stored under MAP at 0°C (Drosinos *et al.*, 1997). Shaw *et al.* (1983) reported that the addition of potassium sorbate to fresh fish inhibited the growth of deteriorating microorganisms, such as *P. flourescens* and *P. fragi* as well as *Alteromonas putrefaciens.* Chung and Lee (1981) demonstrated that

a 1.0% potassium sorbate dip extended lag phase to over 6 days and increased generation times of microflora in English sole homogenate stored aerobically at 1.1°C, but the spoilage pattern (98.2% *Pseudomonas spp.*) was not affected. Kim *et al.* (1995 b) found extension of lag phase of aerobic spoilage bacteria in aerobically stored refrigerated catfish fillets treated with 0.5% sodium acetate (w/w). In the present study significant reduction in *Pseudomonas* counts was observed only in SAVP samples of black pomfret. The results indicated inhibitory effect of acetate on *Pseudomonas*. The results of the present study confirm the earlier findings of Kim *et al.* (1995 b) and Ray (1992) that acetic acid is inhibitory to aerobic spoilage bacteria.

Fig: 33. Changes in counts of *Pseudomonas* of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C

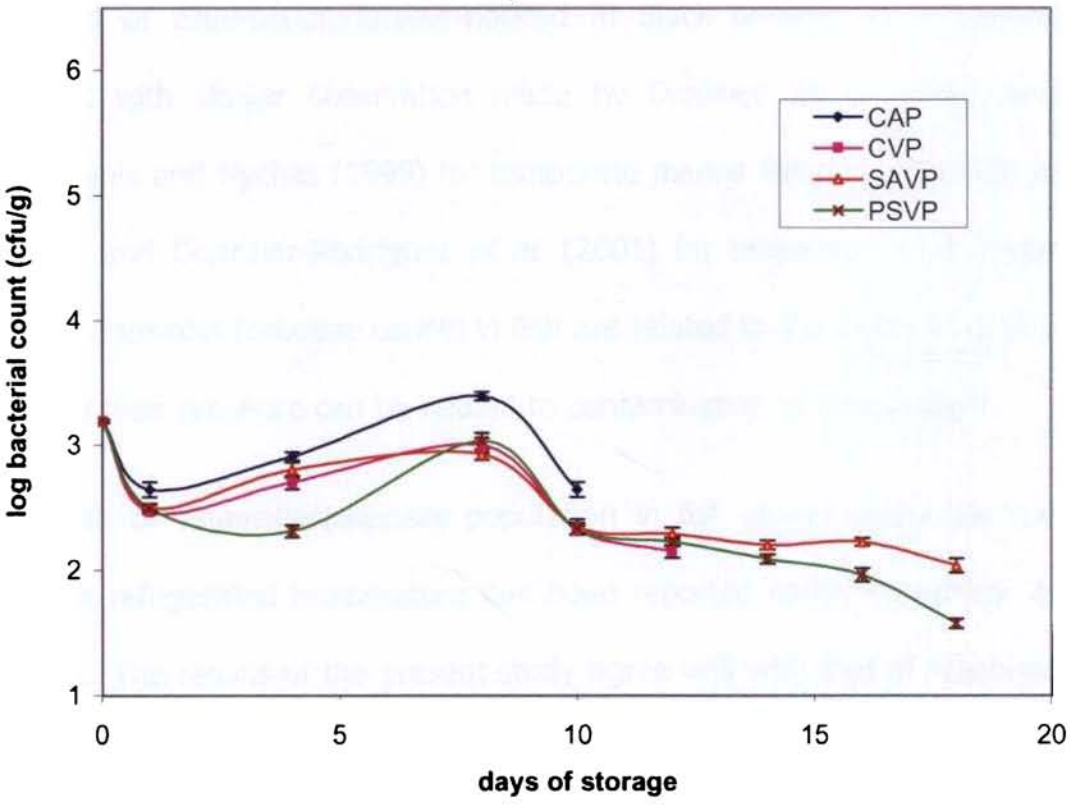


4.8.1.5. Changes in sulphite reducing clostridial count

Fig. 34 illustrates the changes in sulphite reducing clostridia in CAP, CVP, SAVP and PSVP samples of black pomfret stored at 0°C. Initial sulphite reducing clostridial counts were low (3.19 log_{10}cfu/g). Sulphite reducing clostridial counts decreased in all the samples, which may be due to the sensitivity of clostridia to chilling. The results of the present study are in agreement with that of Hobbs (1979). Significant difference was not observed in sulphite reducing clostridial counts between CVP and CAP samples and between SAVP and PSVP samples of black pomfret.

It is generally accepted that sulphite reducing clostridia are sensitive to chilling and freezing (Labbe, 1989). Tompkin *et al.* (1974) did not find any inhibitory effect of potassium sorbate on *C. perifrigens.* Treatments with potassium sorbate and sodium acetate had no additional inhibitory effect in reducing sulphite reducing clostridia.

Fig: 34. Changes in sulphite reducing clostridial counts of black pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.8.1.6. Changes in *Enterobacteriacea, Staphylococcus aureus* and *faecal streptococci* populations

The *Enterobacteriacea* population increased from an initial level of 2.9 log_{10}cfu/g to 4.35, 4.83, 3.48 and 4.67 log_{10}cfu/g respectively in CAP, CVP, SAVP and PSVP samples of black pomfret. Inhibition of growth was noticed in SAVP samples possibly due to the preservative action of sodium acetate. The initial level of *Enterobacteriaceae* noticed in Black pomfret is in general agreement with similar observation made by Drosinos *et al.* (1997) and Koutsoumanis and Nychas (1999) for temperate marine fish and Savvaidis *et al.* (2002) and Gonzalez-Rodriguez *et al.* (2001) for temperate fresh water fish. The *Enterobacteriaceae* counts in fish are related to the hygienic quality of fish and their presence can be related to contamination of faecal origin.

The growth of *Enterobacteriaceae* population in fish stored under air and vacuum at refrigerated temperature has been reported earlier (Arashisar *et al.*, 2004). The results of the present study agree well with that of Arashisar *et al.* (2004). Treatment with 2% sodium acetate significantly inhibited growth of *Enterobacteriaceae* in SAVP samples. Jay (1986) and Zhuang *et al.* (1996) attributed the antimicrobial activity of sodium acetate to undissociated acetic acid molecules and to a reduction of pH below which growth of many bacteria is inhibited.

Drosinos *et al.* (1997) found significant reductions in *Enterobacteriaceae* population in gilt head seabream fillets treated with 5% potassium sorbate.

In contrast, in the present study, 2% potassium sorbate treated black pomfret exhibited a slightly higher count (4.67 log_{10}cfu/g). The results of the study suggest that sodium acetate is more effective in controlling multiplication of *Enterobacteriaceae* in vacuum packed black pomfret.

Staphylococcus aureus count was ca.1.4 log_{10}cfu/g initially in fresh black pomfret and the count decreased or almost remained constant (1.06 log_{10}cfu/g) throughout the storage in CAP, CVP, SAVP and PSVP samples.

Feacal streptococci population showed a little increase (from 3.08 to 3.1-3.2 log_{10}cfu/g) in CAP and PSVP samples while that of CVP and SAVP samples counts decreased by the end of storage. Contrary to this, Al-Dagal and Bazaraa (1999) observed a slight increase in *Streptococcus* counts (4.5 to 5.2%) in whole shrimp treated with sodium acetate (10% w/w) and a decrease in counts (4.4 to 3.6%) in whole shrimp treated with potassium sorbate (1.5% w/w).

4.8.2 Microbiological changes in Pearlspot stored under air and vacuum at 0-2°C

4.8.2.1. Changes in Total viable counts (TVC)

The changes in TVC of CAP, CVP, SAVP and PSVP samples of pearlspot at 20°C and 37°C are shown in figures 35 and 36 respectively. The initial TVC of fresh pearlspot at 20°C and 37°C were 4.98 and 4.94 log_{10}cfu/g respectively. In CAP samples, TVC rose continuously and reached ca.10^7 cfu/g on 10^{th} day. i.e. at the time of sensory rejection. TVC in CVP samples reached the limit count (10^7 cfu/g) on 12^{th} day when the fish were deemed spoiled based on sensory scores. A 1 log increase in the TVC's of treated samples (SAVP and PSVP) was observed on 12^{th} day and the counts increased gradually and reached 10^7 cfu/g on 17^{th} day of storage. Significant difference was observed between TVC's at 20°C and at 37°C at the end of storage.

Bacteria grew most quickly in CAP samples followed by CVP samples of pearlspot. Lowest counts were observed with SAVP (2% w/v) and PSVP (2% w/v) samples. Significant differences were observed between CVP and SAVP and between CVP and PSVP. However, significant difference was not observed between SAVP and PSVP samples.

It has long been known that microbiological counts in fish determined at 20-25°C are much higher than counts determined at 37°C (Castell *et al.*, 1948; Liston, 1957). The TVC of pearlspot at 20°C was higher than that at 37°C in

the present study. Similar observations were also made in Black pomfret. The results of the present study confirm the earlier findings of Castell *et al.* (1948) and Liston (1957).

For fresh fish, the microbiological limit proposed by the ICMSF (1978) is 10^7cfu/g. Since the mean initial counts for Pearlspot were 4.98 $\log_{10}$cfu/g at 20°C, this product could be considered of acceptable microbiological quality. A shelf life of 8 days was obtained for CAP, as it exceeded the acceptability limit on 10^{th} day of storage. Lyhs *et al.* (2001) observed that mesophilic viable counts reached 10^6-10^7 cfu/g at the time of spoilage in vacuum packaged 'gravad' rainbow trout slices during storage at 3°C. CVP samples showed a much slower increase in TVC than CAP, exceeding the acceptability limit only on 12^{th} day of storage. Similar extension of shelf life was also observed for CVP samples of Black pomfret. The results of the study confirm the earlier finding of Leung *et al.* (1992), Huang *et al.* (1994), Lyon and Reddmann (2000) and Özogul *et al.* (2000, 2004) in that bacteria grew most quickly in fish stored in air compared to vacuum pack (VP) at 0°C.

Surface treatments with sodium acetate (2%) and potassium sorbate (2%) was equally effective in inhibiting microbial growth and extending storage life of Pearlspot to 15 days compared to air stored samples (8 days). Similar observations were made in Black pomfret. Significant reduction in TVC observed in treated samples of pearlspot can be attributed to the inhibitory effect of sodium acetate and potassium sorbate on aerobic spoilage bacteria

as reported earlier for catfish fillets (Kim and Hearnsberger, 1994; Zhuang *et al.*, 1996) for *Lethrinus lentjan* fillets (Shalini *et al.*, 2000; 2001) and for silver perch (Gelman *et al.*, 2001).

Fig: 35. Changes in total viable counts (20°C) of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate and untreated) during storage at 0-2°C

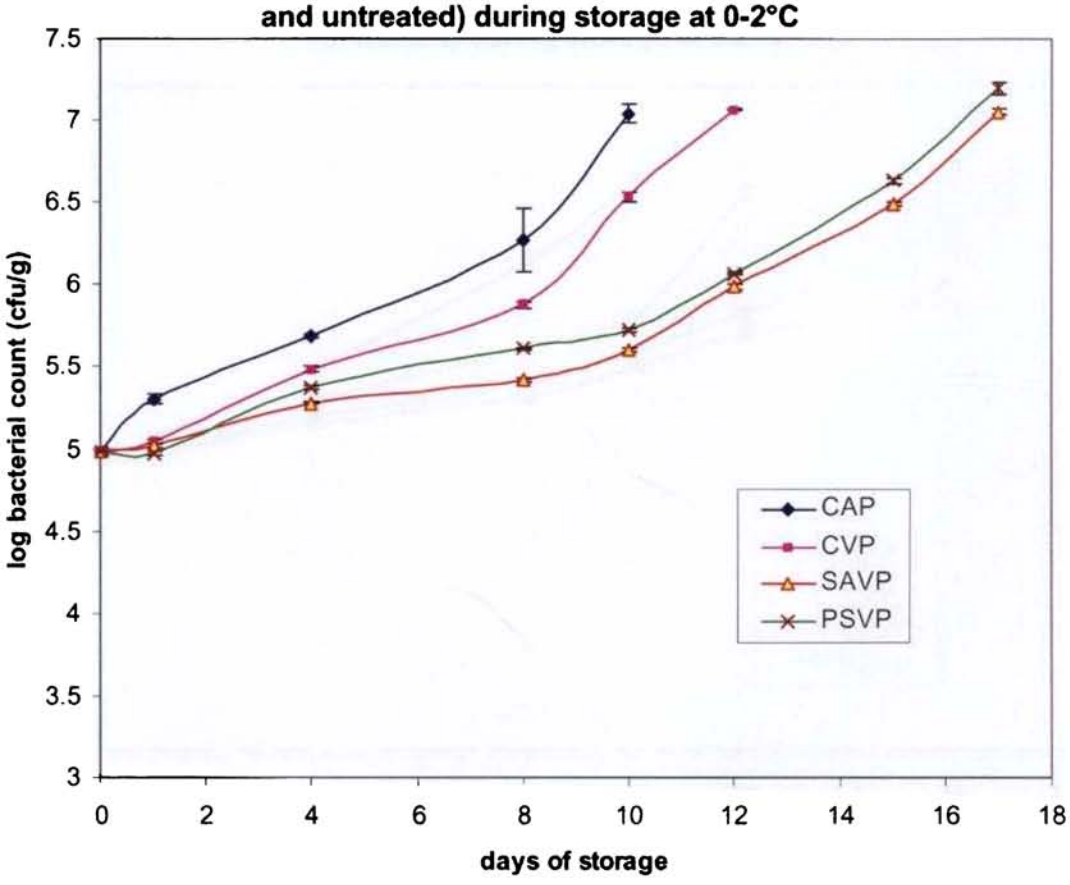
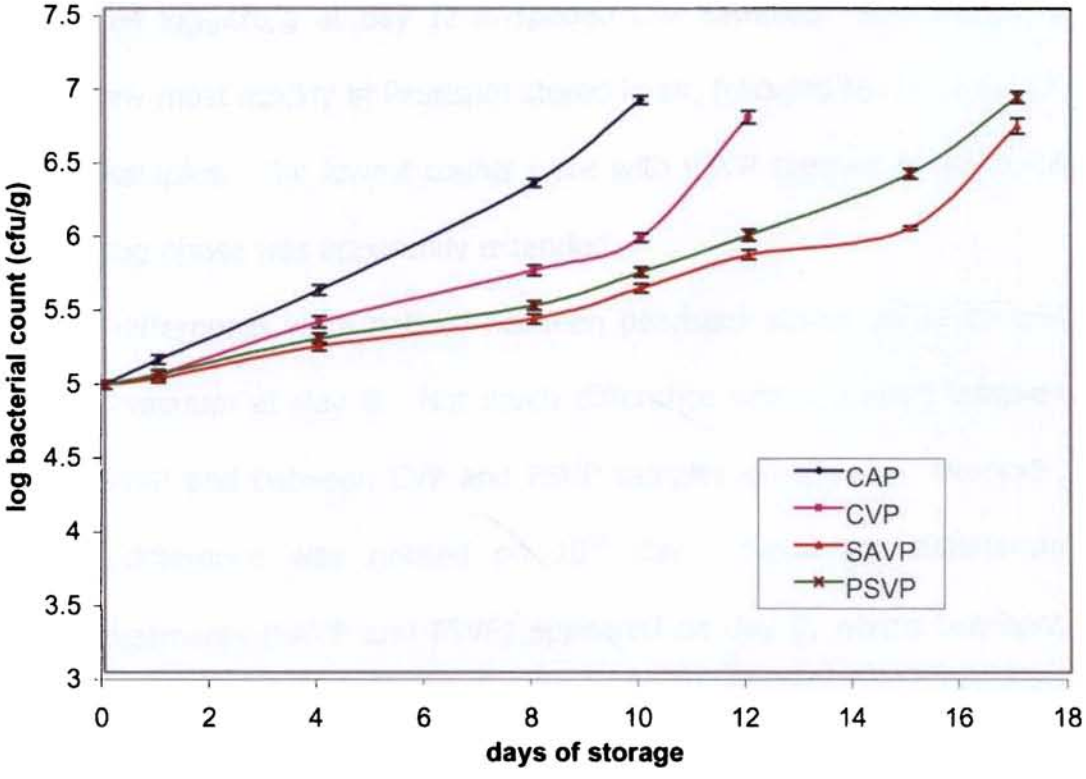


Fig: 36. Changes in total viable counts (37°C) of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate and untreated) during storage at 0-2°C



4.8.2.2. Changes in H_2S producing bacterial count

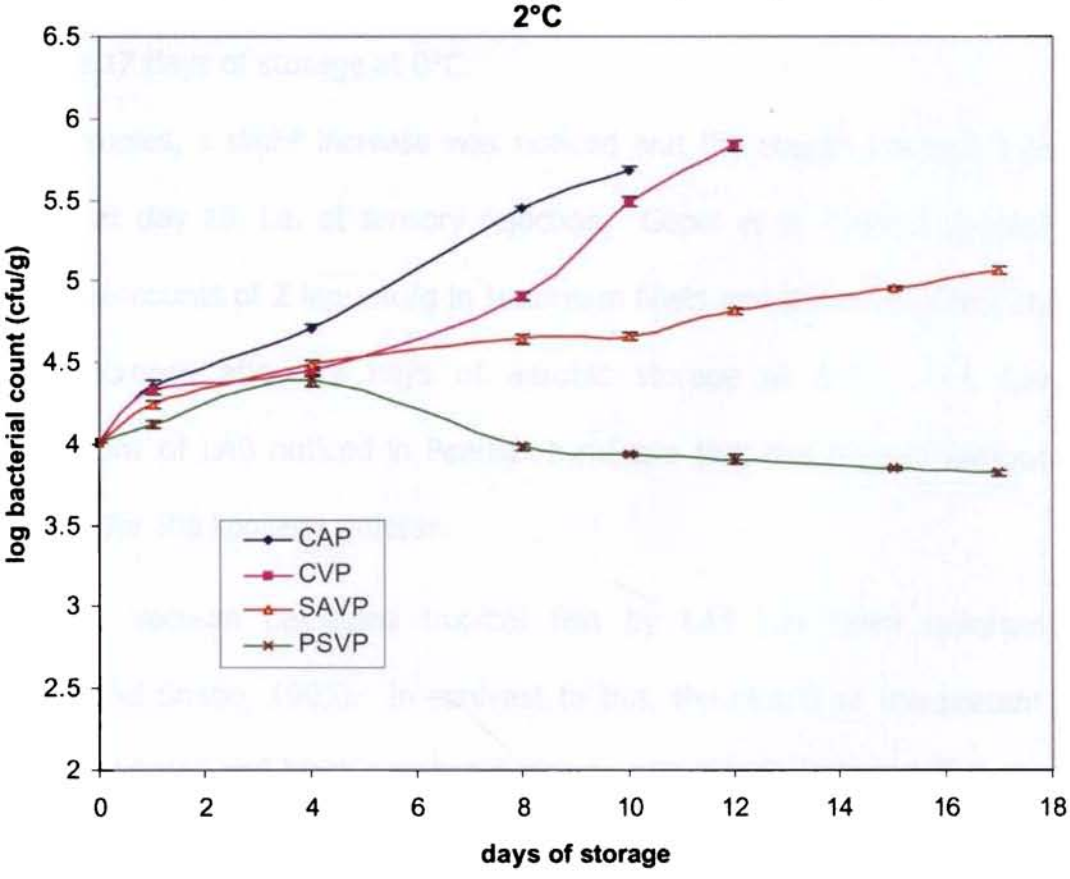
Changes in H_2S producing bacterial counts including *S. putrefaciens* in Pearlspot during storage under air and vacuum at 0-2°C are shown in Fig: 37. H_2S producing bacteria constituted ca.13% of the total flora in fresh pearlspot. They presented very high growth during the first week of aerobic storage and reached 5.78 log_{10}cfu/g at day 10, at which point sensory panelists considered the samples to have reached spoilage. The counts reached 5.84 log_{10}cfu/g at day 12 in spoiled CVP samples. H_2S producing bacteria grew most quickly in Pearlspot stored in air, followed by those in CVP and SAVP samples. The lowest counts were with PSVP samples of pearlspot where the log phase was apparently extended.

Significant differences were noticed between pearlspot stored under air and that under vacuum at day 8. Not much difference was observed between CVP and SAVP and between CVP and PSVP samples on day 4. However, significant difference was noticed on 10^{th} day. Significant differences between treatments (SAVP and PSVP) appeared on day 8, where pearlspot treated with potassium sorbate had lower H_2S producing bacterial populations compared to sodium acetate treated samples. Similar observations were noticed in Black pomfret. The H_2S producing bacterial counts in CVP samples at the time of sensory rejection was ca.10^6cfu/g. These data are in correlation with the results reported by Gram *et al.* (1987) and Jorgensen and Huss (1989).

The initial level of H_2S producing bacteria (4.0 log_{10}cfu/g) reported in this study is comparable to that of Black pomfret and to that of Mediterranean Bouque (Koutsoumanis and Nychas, 1999) and gilt head seabream Drosinos *et al.*, 1987). The number of H_2S producing bacteria at the time of sensory rejection of CAP samples constituted only 5% of the total count where as in CVP samples it constituted 9% of the total count. In contrast to this, H_2S producing bacteria including *S. putrefaciens* constituted 30-40% of the total count in CAP samples and 60-70% of the total count in CVP samples of black pomfret. The results of the present study indicated that H_2S producing bacteria including *S. putrefaciens* is not the main spoilage organism of Pearlspot stored at 0°C.

Several investigators have reported extension of shelf life of seafoods using wide concentration ranges of organic acid salts such as sodium acetate (0.5 to 10% w/w) and potassium sorbate (0.1 to 10%) alone or in combination (Zhuang *et al.*, 1996; Al-Dagal and Bazaraa, 1999; Shalini *et al.*, 2000 and Drosinos *et al.*, 1997). In general, treatment with potassium sorbate significantly suppressed the growth of H_2S producing bacteria during 17 days of storage by extending the log phase. The results of the present study suggest that treatment with potassium sorbate is most effective in suppressing the H_2S producing bacteria in Pearlspot.

Fig: 37. Changes in counts of H_2S producing bacteria in pearlspot packed under air and vacuum (sodium acetate/potassium sorbate and untreated) during storage at 0-2°C



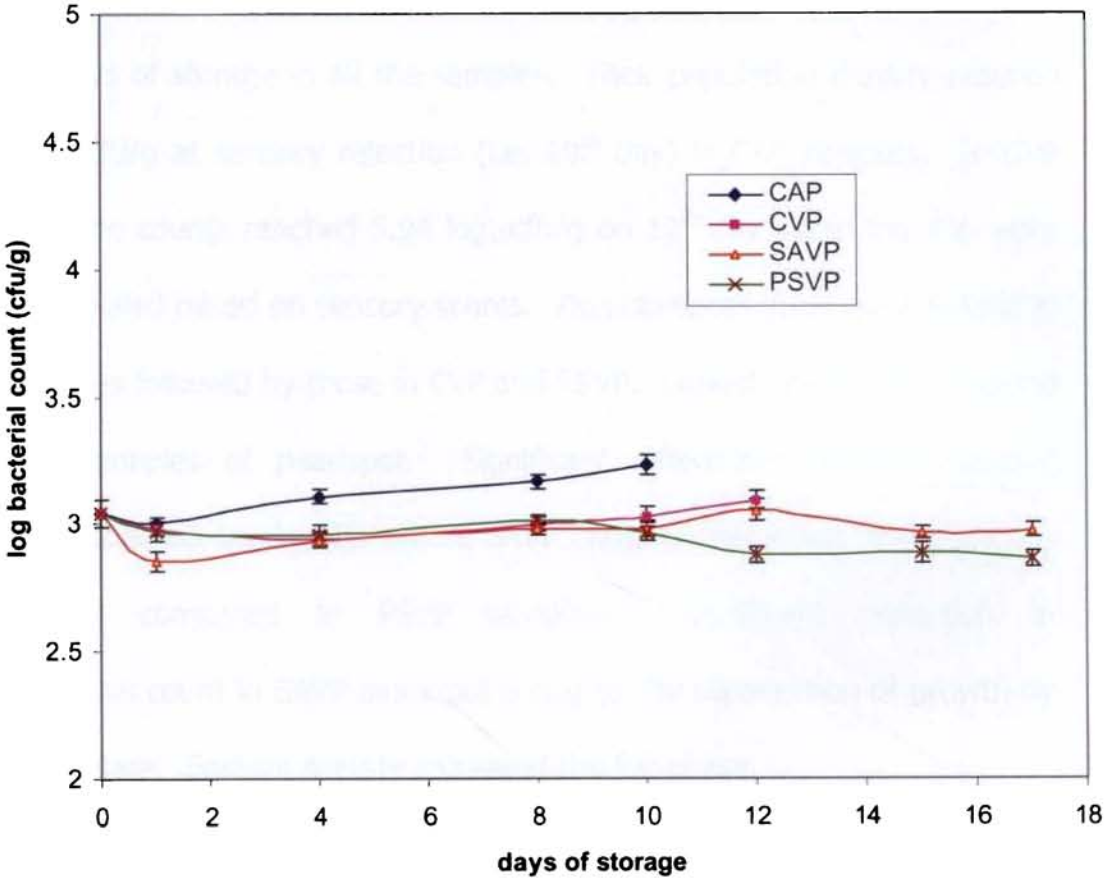
4.8.2.3. Changes in Lactic Acid Bacteria

The changes in LAB in Pearlspot stored under air and vacuum with and without treatments using sodium acetate and potassium sorbate are presented in Fig. 38. The initial LAB populations in Pearlspot were low (3.04 log_{10}cfu/g). LAB did not grew in CVP, SAVP and PSVP samples of pearlspot during the 17 days of storage at 0°C.

In CAP samples, a slight increase was noticed and the counts reached 3.23 log_{10}cfu/g at day 10, i.e. at sensory rejection. Gopal *et al.* (1996) reported *Lactobacillus* counts of 2 log_{10}cfu/g in seabream fillets and they exhibited only a slight increase after 22 days of aerobic storage at 0°C. The low concentrations of LAB noticed in Pearlspot indicate that this flora is without importance for the spoilage process.

Spoilage of vacuum packaged tropical fish by LAB has been reported (Pedersen and Snabe, 1995). In contrast to this, the results of the present study on Pearlspot and black pomfret suggests that this flora is not likely to be responsible for spoilage of air or vacuum packed Pearlspot and Black pomfret.

Fig: 38. Changes in Lactic acid bacterial counts of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



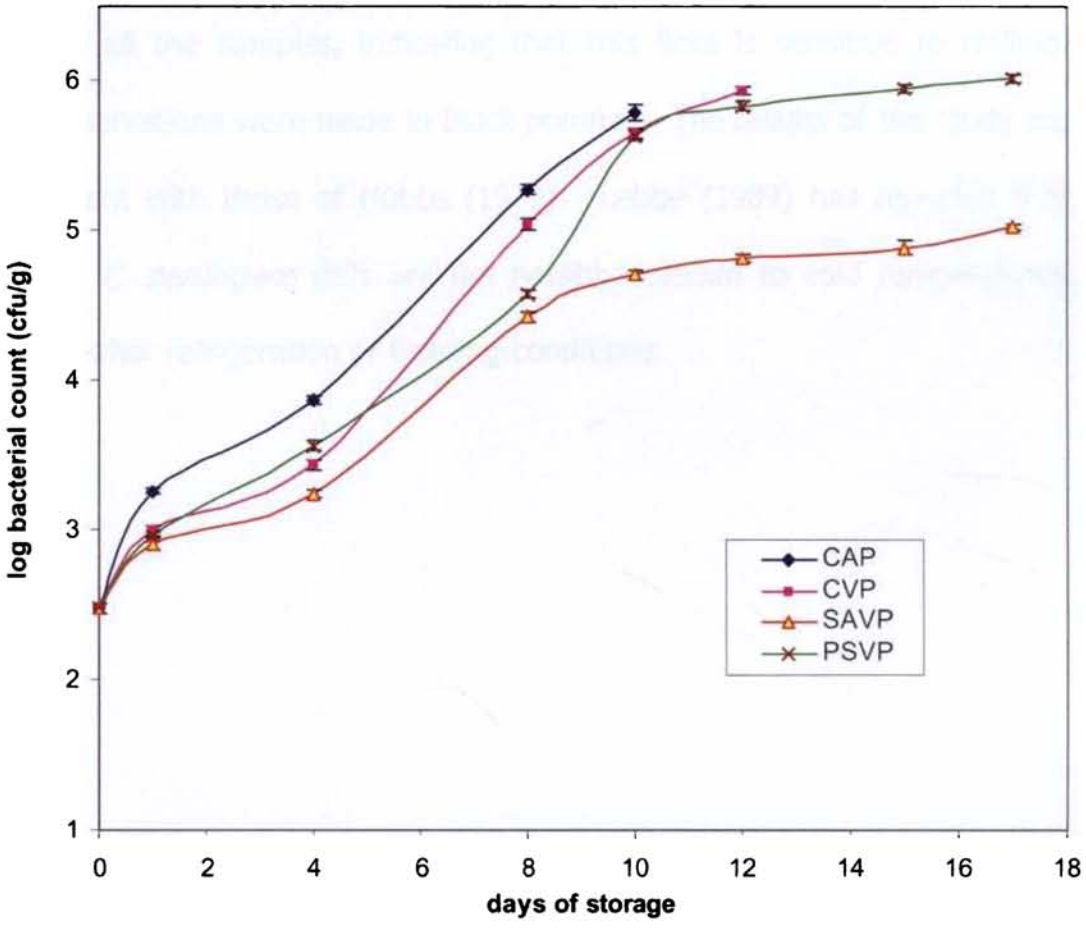
4.8.2.4. Changes in counts of *Pseudomonas*

The initial *Pseudomonas* population in fresh Pearlspot was 2.48 log_{10}cfu/g. The changes in Pseudomonas count in CAP, CVP, SAVP and PSVP samples are shown in Fig. 39. Significant increase in *Pseudomonas* count was noticed after 4 days of storage in all the samples. Their population density reached 5.79 log_{10}cfu/g at sensory rejection (i.e. 10^{th} day) in CAP samples. In CVP samples, the counts reached 5.94 log_{10}cfu/g on 12^{th} day when the fish were deemed spoiled based on sensory scores. *Pseudomonas* grew most quickly in CAP samples followed by those in CVP and PSVP. Lowest counts were noticed in SAVP samples of pearlspot. Significant differences between treated samples appeared on day 10, where SAVP samples had lower *Pseudomonas* populations compared to PSVP samples. Significant reduction in *Pseudomonas* count in SAVP pearlspot is due to the suppression of growth by sodium acetate. Sodium acetate increased the log phase.

Pseudomonas population constituted <1% of the total flora of fresh Pearlspot. By the end of storage, 8-9% of the total flora was constituted by *Pseudomonas* in CAP and CVP samples respectively. In PSVP samples, still higher percentage (10%) was noticed. *Pseudomonas* together with *S. putrefaciens* are reported to be the spoilers of marine tropical fish stored in ice (Gram, 1992; Koutsoumanis and Nychas, 1999). Investigations on black pomfret also suggest that these two species of bacteria are the spoilers. The results of this study indicate that *Pseudomonas* together with *S. putrefaciens*

are the spoilers of Pearlspot. The antimicrobial effects of sodium acetate and potassium sorbate on the aerobic spoilage bacteria of fish has been documented (Kim *et al.*, 1995 b). Al-Dagal and Bazaraa (1999) reported that sodium acetate was significantly more effective in suppressing psychrotrophic bacterial growth than potassium sorbate along with bifidobacteria. In the present study, sodium acetate was more effective in suppressing *Pseudomonas* population compared to potassium sorbate.

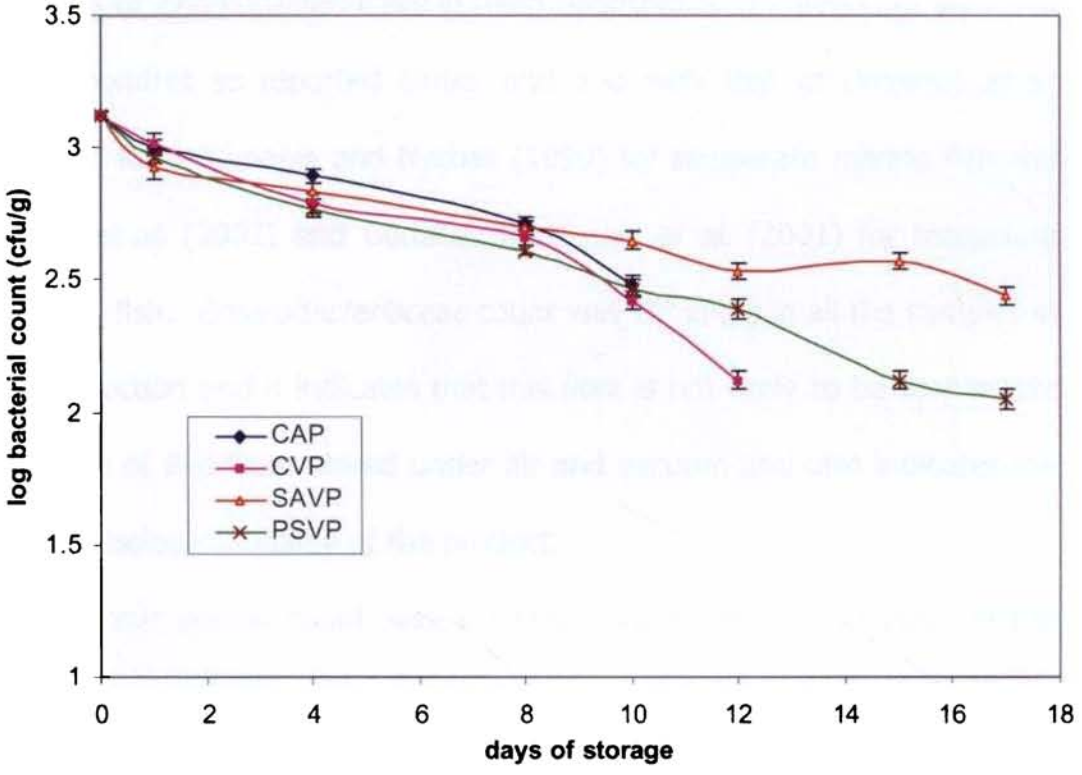
Fig: 39. Changes in counts of *Pseudomonas* of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.8.2.5. Changes in sulphite reducing clostridial count

Sulphite reducing clostridial counts were low initially (3.12 $\log_{10}$cfu/g). The changes in sulphite reducing clostridial population in Pearlspot stored under air and vacuum at 0-2°C with and without sodium acetate and potassium sorbate treatment is shown in Fig. 28. Their counts were found to decrease during storage in all the samples, indicating that this flora is sensitive to chilling. Similar observations were made in Black pomfret. The results of the study are in agreement with those of Hobbs (1979). Labbe (1989) has reported that vegetative *C. perifrigens* cells are not notably tolerant to cold temperatures including either refrigeration or freezing conditions.

Fig: 40.Changes in sulphite reducing clostridial counts of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0-2°C



4.8.2.6. Changes in *Enterobacteriacea, Staphylococcus aureus* and *faecal streptococci* populations

The counts of *Enterobacteriacea* in fresh pearlspot were 2.81 log_{10}cfu/g. By the end of storage, a 0.2 log increase was noticed in CVP, SAVP and PSVP samples whereas in CAP samples of pearlspot 0.4 log increase was observed. The counts of *Enterobacteriaceae* in fresh Pearlspot is in agreement with that of Black pomfret as reported earlier and also with that of Drosinos *et al.* (1997) and Koutsoumanis and Nychas (1999) for temperate marine fish and Savvaidis *et al.* (2002) and Gonzalez-Rodriguez *et al.* (2001) for temperate fresh water fish. *Enterobacteriaceae* count was 10^3 cfu/g in all the samples at sensory rejection and it indicates that this flora is not likely to be responsible for spoilage of Pearlspot stored under air and vacuum and also indicates the good microbiological quality of the product.

Staphylococcus aureus count was 1.2 log_{10}cfu/g in fresh pearlspot. On the day of sensory rejection, the counts decreased (0.2 log reduction) in all the samples. The counts were within the limit prescribed for fresh fish by ICMSF (1986).

Faecal streptococci population was 2.58 log_{10}cfu/g in fresh Pearlspot. A 0.2-0.25 log reduction in *faecal streptococcal* counts were noticed in CAP, CVP and SAVP samples. In PSVP pearlspot, 0.3 log reduction was found. A similar reduction in *streptococcus* counts was noticed earlier for whole shrimp treated with 1.5% potassium sorbate (Al-Dagal and Bazaraa, 1999). The results of

the study indicate good microbiological quality of fresh Pearlspot and Pearlspot samples stored at 0-2°C.

4.8.3. *C. botulinum* toxin detection by mouse bioassay

Clostridium botulinum toxin was not detected in any of the samples throughout the storage period, which indicates that there was no abuse of temperature during storage. This negative result for *C. botulinum* toxin assay obtained is in accordance with work reported by Lilly and Kautter (1990) for vacuum packed fish fillets, modified atmospheric packed salmon fillets by Reddy *et al.* (1997a) and modified atmospheric packed fresh catfish fillets by Reddy *et al.* (1997b).

CODE OF PRACTICE

5.0. CODE OF PRACTICE

National Advisory Committee on microbiological criteria for foods (NACMCF) report on Vacuum Packaging for Refrigerated Raw Fishery Products (1992)

NACMCF examined microbiological safety issues associated with vacuum packaging of refrigerated raw fishery products. They are as follows.

1. Raw fish quality

Prior to packaging proper handling of raw fish must be assured from the point of harvest. Vacuum packaging must not be used to extend the shelf life of fish whose quality has deteriorated.

2. Hazard Analysis Critical Control Point (HACCP) Plan.

A HACCP plan from point of harvest till it is marketed must be developed for vacuum packaging, recognizing that rigid temperature control is the primary preventive measure to ensure safety.

3. Hazard Analysis/ Risk Assessment.

The studies to support hazard analysis/risk assessment must be completed by food safety experts who are competent in HACCP systems, *Clostridium botulinum* methodology, sensory evaluations, and statistical procedures.

The evaluated system cannot be used for marketing fish unless both the experimental results and the statistical modeling demonstrate that odour rejection will always precede toxin production. If this is not demonstrated,

i.e., if toxin production precedes odour rejection, vacuum packaging technology cannot be used for the species of fish studied.

No new study to demonstrate the efficacy and safety of the process is necessary as long as the data from the basic study can be applied. A new study, including the inoculated pack and shelf life, however, is necessary when different species of fish are used, a new ingredient is added or the distribution systems or targeted users have substantially changed.

The studies to support hazard analysis/ risk assessment of vacuum packaging include the following:

A. Process Description

A HACCP – based description of the species of fish investigated and the handling procedures must be developed.

B. Inoculated Pack Studies

Inoculated pack studies must be performed for each type of fish and packaging technology to confirm the efficacy and safety and to guide the development of an appropriate distribution strategy. All studies must be conducted in a facility that has the safeguards in place to work with *Clostridium botulinum.*

C. Sensory Evaluation

A sensory evaluation method using specific odour rejection criteria to determine the onset of spoilage must be selected, standardized, and accepted.

D. Statistical Evaluation.

Predictive statistical models must be based on the experimental data to evaluate the potential of toxin production before odour rejection.

4. System Design Validation

The results of the inoculated pack and sensory evaluation study can be used to determine whether the system design is effective and safe. Modify the processing system where necessary. Perform additional inoculated pack and sensory evaluation studies if the system is modified. Validate the HACCP plan. The total processing system and associated HACCP system must be approved by food safety experts competent in assessing the potential food safety hazards.

5. Labeling Requirements

Labeling requirements must include:

A. Keep refrigerated at 3.3 °C or below.

B. Use by date.

C. Cook fully before serving.

The use-by date must be experimentally determined, and should be a date at which product quality is still maintained.

6. Time and Temperature Records.

Time/temperatures recorders should be enclosed in selected sealed master cartons during shipment of seafood products at the time each shipment is received, the individual time temperature charts should be examined and the shipment or carton rejected if the product temperature exceeds 3.3°C.

Technology, reliability, and costs of time/temperature integrators are to be developed to the point where their use becomes very practical.

Recommended practice for vacuum packed products.

1. The restricted use of vacuum packaging for refrigerated raw fishery products can be considered only if sufficient safeguards detailed in an established HACCP plan can be implemented and verified to control the specific safety concerns regarding the product.
2. Vacuum packaging technology is to be permitted only when it is assured that detectable spoilage and rejection by the consumer precedes the possibility of toxin production.
3. Vacuum packaging should be used only with high quality raw fish. It must not be used to extend the shelf life of fish whose quality has deteriorated.
4. Vacuum packed products must be held at or below 3.3°C at all points from packaging through final preparation. This temperature requirement must be clearly indicated on shipping cartons and retail package labels. There should not be any abuse of temperature during storage and transportation.
5. Secondary measures in addition to refrigeration must be employed to increase assurance of product safety. These include additional labeling requirements and stringent processing controls.
6. Vacuum packaging technology must ensure that the intended vacuum is achieved with appropriate films.

7. The minimum conditions for vacuum packaging technology as described above must be followed.

8. In India it can be effectively applied for highly priced table fishes like seer, pomfret, pearlspot etc.

SUMMARY AND CONCLUSIONS

6.0. SUMMARY AND CONCLUSIONS

Fish plays a very vital role in human nutrition and is the primary source of animal protein for over one billion people of developing countries. About 85-90% of fish protein are easily digestible and contain all essential amino acids. In a developing country like India, where frozen fish industry for internal marketing may not flourish in near future due to lack of facility of cold chain, the preservation of fish by icing will play a vital role in the distribution of fish. Bulk transportation of fresh fish in ice provides only a limited extension of shelf life. Since the fish are not surface protected by over wrapping or other packaging cross contamination, leaching of soluble nutrients and other forms of abuse cannot be avoided.

In today's affluent society, people prefer to buy ready-to-cook and ready-to-serve convenience products from supermarkets than buying raw fish. Quality issues are vital for every industrial sector in today's market. Especially in the food industry, quality is a primal condition in any phase within the food-processing chain. Consumers became very conscious and define and judge food quality by either visual or physical quality. These phenomena in the international food chain markets require also new requirements and demands for food packaging. In the first place, food should be able to be stored or distributed for longer periods. Packaging can differentiate the food in respect of freshness, taste or even image. One of the major developments in food packaging for fulfilling the new challenges is packaging under vacuum or

modified atmosphere conditions. Vacuum packaging suits the demands and requirements of today and tomorrow.

Vacuum packaging, a technologically viable method has been developed as a supplement to ice or mechanical refrigeration to reduce losses and to extend the storage life of fresh seafood products. In vacuum packaging, the product is contained in a package made of a material having low oxygen permeability and is sealed airtight after evacuating the air. It fits into an important area of preservation where shelf life is extended without the loss of those important and exclusive properties, which constitute freshness in consumer's mind, and therefore move the product into a premium bracket.

Preservatives such as sodium acetate and potassium sorbate are found to be effective in preventing microbial growth and improving shelf life under different storage conditions. However, collective works on various quality aspects of vacuum packaged fish of tropical region, under refrigerated storage are found to be scarce. So, the present work was carried out to study the effect of vacuum packaging on the shelf life of fresh pearlspot and black pomfret during chill storage and also the effect of chemical preservatives such as sodium acetate and potassium sorbate in extending the shelf life. Pearlspot and Black pomfret are highly cherished food fishes of India and have good export market.

Fresh pearlspot and black pomfret from Fort kochi fish market were brought to the laboratory in iced condition for further processing. Both the species were beheaded, scaled, gutted, washed in potable water and made into

steaks in case of Black pomfret. Fish samples were given a dip treatment in 2 ppm chilled chlorinated water for 10 min. and drained well. Pearl spot/black pomfret steaks were divided into 4 lots. Control Air Pack (CAP), Control Vacuum Pack (CVP), Sodium acetate (2% w/v) treated vacuum pack (SAVP) and Potassium sorbate (2% w/v) treated vacuum pack (PSVP) to study the effect of vacuum packaging with and without treatment in comparison to air packed samples.

Pouches made of 12μ polyester laminated with 300 gauge low density polyethylene were used for packing Pearlspot/black pomfret steaks. The physical properties of the packaging material studied include Tensile strength, Elongation at break and Heat seal strength which were determined in both machine direction and cross direction. Water vapour transmission rate and oxygen transmission rate of the packaging material were measured to be 3.62 $g/m^2/24h$ at 37°C and 90±2% RH and $65cc/m^2/atmosphere/24hrs$ at room temperature respectively. The overall migration residue (water extractives) at 21.1°C for 48 hrs of the sample was found to be 3.35 mg/l, which is below the acceptable limit for food contact application. The values indicate that the packaging material meet the requirements for vacuum packed products. Pearlspot/Black pomfret samples after treatment and packing were stored in ice and kept in a chill room maintained at 0-2°C. Samples were subjected to biochemical, textural, microbiological, and sensory evaluation at regular intervals. The changes during chill storage in these samples are discussed below.

In case of pearlspot drip loss increased from an initial value of 2.63% on 4th day to 4.92% on 10th day in CAP, 3.52 to 6.43% on 12th day in CVP, 3.1 to 8.92% in SAVP and 3.66 to 9.25% in PSVP samples on 17th day of storage. In black pomfret, drip was found to increase from an initial value of 1.92% on 4th day to 2.23% on 10th day in CAP, 2.61 to 6.71% on 12th day in CVP, 2.34 to 6.70% in SAVP and 2.97 to 7.05% in PSVP samples on 18th day of storage. Drip loss was observed to be more in vacuum packed samples compared to treated samples. In both the species, air packed samples exhibited minimum drip loss.

TVB-N values increased gradually in all the samples during storage. In pearlspot, value increased from initial 5.6 mg% to 19.45, 18.3, 21.6 & 17.15 mg% in CAP, CVP, SAVP & PSVP samples on 10th, 12th and 17th day of storage respectively. The TVB-N values of treated samples were found to be comparatively lesser than those of control packs. In black pomfret, the value increased from initial 4.5 mg% to 19.6, 18.4, 21.55 & 16.65 mg% in CAP, CVP, SAVP & PSVP samples on 10th, 12th and 18th day of storage respectively. In both the species, TVB-N contents of potassium sorbate treated samples were slightly lower than sodium acetate treated samples. This might be attributed to the greater inhibition of aerobic gram -ve bacteria by potassium sorbate than sodium acetate. However, TVB-N values in all the samples were within the suggested limit throughout the storage period.

A gradual increase was observed in TMA-N values of all the samples during storage. In pearlspot, value increased from initial 1.4 mg% to 6.55, 6.0, 6.1 &

5.25 mg% in CAP, CVP, SAVP & PSVP samples on 10^{th}, 12^{th} and 17^{th} day of storage respectively. The TMA-N values of treated samples are found to be comparatively lesser than those of control packs in both the species. In black pomfret, the value increased from initial 2.8 mg% to 15.8, 16.1, 16.35 & 14.25 mg% in CAP, CVP, SAVP & PSVP samples on 10^{th}, 12^{th} and 18^{th} day of storage respectively. In case of pearl spot, TMA-N values were within the limit till the end of storage, whereas in case of Black pomfret the values exceeded the acceptability limit in control as well as sodium acetate treated packs on the day of sensory rejection.

An increasing trend in the TBA values was noticed in all the samples with storage time. The initial TBA value in Pearlspot and Black pomfret was found to be 0.08 and 0.28 mg malonaldehyde/kg of fish respectively. On the day of sensory rejection, the values rose to 0.31, 0.34, 0.39 and 0.31 in CAP, CVP, SAVP and PSVP samples respectively in case of pearlspot. In black pomfret, TBA values on the day of sensory rejection were found to be 1.85, 1.33, 1.14 and 1.13 in CAP, CVP, SAVP and PSVP samples respectively. In both the species, vacuum packed and treated samples showed lower value in comparison to air packed samples. Potassium sorbate treated samples exhibited still lower TBA values than those of sodium acetate treated samples. In all the samples, TBA values were within the limit through out the storage period.

Pearlspot and black pomfret had an initial pH of 6.5 and 6.47 respectively. The pH values were found to increase gradually during storage in air packed

samples of both the species. In CVP, SAVP and PSVP samples of both the species, the values exhibited a slight decrease initially and then increased. Slight decrease in pH values may be attributed to the dissolution of CO_2 in the fish muscle. Increase in pH may be attributed to the production of volatile basic compounds by bacterial activity. On the day of sensory rejection, the pH values of pearlspot were found to be 6.58, 6.56, 6.56 and 6.58 in CAP, CVP, SAVP and PSVP samples respectively. In black pomfret, the values in CAP, CVP, SAVP and PSVP samples on the day of sensory rejection were found to be 6.59, 6.54, 6.55 and 6.52 respectively.

In both the species, K value was found to increase during storage. In pearlspot, K value increased from initial 4.87% to 68.7, 61.01, 68.39 and 69.4% in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, the value increased from initial 7.81% to 67.13, 63.69, 63.43 and 62.17% on the day of sensory rejection. K values of all the samples have exceeded 60% on the day of sensory rejection, which indicates good correlation of K value with the sensory scores.

Textural parameters studied involve changes in hardness1 and hardness2 values, cohesiveness, springiness and chewiness of fish muscle during storage. In both the species, Hardness 1 and Hardness 2 values were found to decrease during storage. In pearlspot, hardness 1 decreased from an initial value of 1.99 kgf to 1.86, 1.60, 1.54 and 1.47 kgf in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, Hardness 1 decreased from initial 1.51 kgf to 0.92, 1.05, 0.99 and 0.86 kgf in

CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection.

Hardness 2 decreased from initial 1.77 kgf to 1.63, 1.40, 1.32 and 1.25 kgf in CAP, CVP, SAVP and PSVP samples of pearlspot respectively on the day of sensory rejection. In black pomfret, hardness 2 decreased from initial 1.26 kgf to 0.88, 0.83, 0.74 and 0.72 kgf in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. Decrease in hardness 1 and hardness 2 values might be attributed to the weakening of connective tissue of fish muscle during storage.

Significant change was not observed in cohesiveness of both the species during storage. In pearlspot, cohesiveness slightly decreased from initial 0.34 to 0.28, 0.26, 0.22 and 0.22 in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, also a slight decrease was observed from initial 0.32 to 0.28 and 0.27 in CAP and CVP samples respectively and to 0.26 in SAVP and PSVP samples. This indicates that there was not much change in the internal bonding of fish muscle during storage.

A decreasing trend was observed in the springiness of both the species during storage. In Pearlspot, springiness decreased from initial 1.01mm to 0.92, 0.86, 0.75 and 0.68 mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, the values decreased from initial 2.72mm to 2.66, 2.45, 2.10 and 2.41mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection.

Chewiness was found to decrease in both the species during storage. In Pearlspot, chewiness decreased from initial 0.65 kgf.mm to 0.48, 0.26, 0.24 and 0.23 kgf.mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, the values decreased from initial 1.35 kgf.mm to 0.85, 0.73, 0.64 and 0.53 kgf.mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. Decrease in chewiness indicates that the fish muscle becomes soft during storage.

In both the species, a significant decline in sensory score was observed in control and treated packs during storage. A sensory score of 4 was taken as the borderline of acceptability. In Pearlspot, sensory scores declined from an initial 8.6 to 3.4, 3.8, 3.6 and 3.8 in CAP, CVP, SAVP and PSVP samples 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. Thus, vacuum packaging alone was not much effective in extending the shelf life of pearl spot, but vacuum packaging along with preservatives (sodium acetate / potassium sorbate) was found to extend the shelf life by 7 days compared to air packed samples.

In black pomfret, the sensory scores declined from an initial 8.8 to 3.4, 3.8, 3.6 and 3.8 in CAP, CVP, SAVP and PSVP samples respectively on the day of rejection. Thus, air packed and vacuum packed samples were acceptable up to 8 days and 10 days respectively. As in case of pearlspot, an extension of only 2 days was noticed due to packing under vacuum. SAVP and PSVP samples remained in good condition up to 16 days. Thus, an extension of 8

days in shelf life was obtained by the combination of vacuum packaging along with preservatives (sodium acetate and potassium sorbate) compared to air packed samples, which were acceptable only up to 8 days. In both the species, significant difference was not noticed between sodium acetate and potassium sorbate treated samples.

Microbiological parameters studied include, changes in total viable count (TVC) at 20°C and 37°C, H_2S producing bacterial count, *Pseudomonas*, Lactic Acid Bacteria, sulphite reducing clostridial count and *Clostridium botulinum* toxin detection by mouse bioassay.

The initial total viable counts at 20°C and 37°C in black pomfret were 5.48 and 5.52 log_{10}cfu/g respectively. In CAP samples, TVC exceeded 10^7 cfu/g on 10^{th} day when they were sensorily rejected. In CVP samples, after a lag phase of 8 days significant increase in TVC was noticed and the limit count of 10^7 cfu/g was reached on 12^{th} day of storage. The lowest counts were noticed in SAVP and PSVP samples where the log phase was apparently extended. The initial total viable counts at 20°C and 37°C did not differ significantly. At the end of storage, counts at 20°C were significantly higher than that at 37°C and it indicates that considerable proportion of the bacteria easily got adapted to grow at low temperature. Based on TVC's shelf life of 16 days was noticed for both SAVP and PSVP samples compared to 10 days for CVP and 8 days for CAP samples.

The initial TVC of fresh pearlspot at 20°C and 37°C were 4.98 and 4.94 log_{10}cfu/g respectively. In CAP samples and CVP samples, TVC rose

continuously and reached ca.10^7 cfu/g on 10^{th} and 12^{th} day of storage respectively when the fish were deemed spoiled based on sensory scores. A 1 log increase in the TVC's of treated samples (SAVP and PSVP) was observed on 12^{th} day and the counts increased gradually and reached 10^7 cfu/g on 17^{th} day of storage. Surface treatments with sodium acetate (2%) and potassium sorbate (2%) was equally effective in inhibiting microbial growth and extending storage life of Pearlspot to 15 days compared to 10 days for CVP samples and 8 days for air stored samples.

The initial concentration of H_2S producing bacteria in Black pomfret was 10^3-10^4 cfu/g and it constituted 1% of the total viable counts at day 0. In CAP and CVP samples, H_2S producing bacteria proliferated and the counts reached ca. 10^7 cfu/g respectively on 10^{th} and 12^{th} day when the samples were sensorily rejected. The H_2S producing bacterial counts reached 5.26 and 4.65 log_{10}cfu/g in SAVP and PSVP samples of black pomfret respectively when they were rejected based on sensory scores. Proliferation of H_2S producing bacteria was prevented by treatment with potassium sorbate. The number of H_2S producing bacteria at rejection of vacuum packed samples constituted 60-70% of the total count while that of CAP was 30-40%. *S. putrefaciens* was identified as the main spoilage organism of black pomfret. Treatments with sodium acetate and potassium sorbate suppressed the growth of H_2S producing bacteria by extending the log phase and thereby extending shelf life.

The initial level of H_2S producing bacteria in Pearlspot was 4.0 log_{10}cfu/g. The counts reached 5.78 and 5.84 log_{10}cfu/g in CAP and CVP samples on 10^{th} and 12^{th} day of storage respectively, when the samples were rejected based on sensory scores. H_2S producing bacteria grew most quickly in Pearlspot stored in air, followed by those in CVP and SAVP samples. The lowest counts were with PSVP samples of pearlspot where the log phase was apparently extended. H_2S producing bacteria at the time of sensory rejection of CAP samples constituted only 5% of the total count where as in CVP samples it constituted 9% of the total count.

The initial level of Lactic acid bacteria in black pomfret was 3.47 log_{10}cfu/g. The LAB count decreased or remained almost constant in CAP and CVP samples. In SAVP and PSVP samples, their count decreased in the first two weeks of storage. Their population density was always <3.8 log_{10}cfu/g in SAVP and PSVP samples whereas in CAP and CVP samples it was 3.29 -3.31 log_{10}cfu/g at the time of sensory rejection. In Pearlspot the initial LAB populations were low (3.04 log_{10}cfu/g) and it did not grew in CVP, SAVP and PSVP samples of pearlspot during the 17 days of storage at 0°C. In CAP samples, a slight increase was noticed and the counts reached 3.23 log_{10}cfu/g at day 10, i.e. at sensory rejection. The results of the present study on Pearlspot and black pomfret suggests that this flora is not likely to be responsible for spoilage of air or vacuum packed Pearlspot and Black pomfret.

In CAP and CVP samples of black pomfret, *Pseudomonas* count increased from initial 2.05 log_{10}cfu/g and reached 5.1 log_{10}cfu/g and 4.45 log_{10}cfu/g

respectively when they were sensorily rejected. In PSVP samples, population density gradually increased and reached 4.23 log_{10}cfu/g on 18th day of storage at 0°C. Significant reduction in *Pseudomonas* count was observed in SAVP samples of black pomfret where the log phase was apparently extended.

The initial *Pseudomonas* population in fresh Pearlspot was 2.48 log_{10}cfu/g and constituted <1% of total flora of fresh pearlspot. Their population density reached 5.79 and 5.94 log_{10}cfu/g in CAP and CVP samples respectively when the fish were deemed spoiled based on sensory scores. Lowest counts were noticed in SAVP samples of pearlspot. By the end of storage, 8-9% of the total flora was constituted by *Pseudomonas* in CAP and CVP samples respectively. In PSVP samples, still higher percentage (10%) was noticed. The results indicate that *Pseudomonas* together with *S. putrefaciens* constitutes the spoilers in both the species. Sodium acetate was more effective in suppressing *Pseudomonas* population compared to potassium sorbate.

In both the species sulphite reducing clostridial counts were low initially. Initial count in Pearlspot and black pomfret were 3.12 log_{10}cfu/g and 3.19 log_{10}cfu/g respectively. Decrease in counts was observed in both the species during storage, which indicated that the flora is sensitive to chilling. Treatment with potassium sorbate and sodium acetate had no additional inhibitory effect in reducing sulphite reducing clostridia in both the species.

Counts of *Staphylococcus aureus, Faecal Streptococci* and *Enterobacteriacea* were determined for fresh fish and for fish at the time of sensory rejection. The *Enterobacteriacea* population increased from an initial level of 2.9 log_{10}cfu/g to 4.35, 4.83, 3.48 and 4.67 log_{10}cfu/g respectively in CAP, CVP, SAVP and PSVP samples of black pomfret. Inhibition of growth was noticed in SAVP samples possibly due to the preservative action of sodium acetate. *Staphylococcus aureus* count was about 1.4 log_{10}cfu/g initially in fresh black pomfret and the count decreased or almost remained constant (1.06 log_{10}cfu/g) throughout the storage in CAP, CVP, SAVP and PSVP samples. *Feacal streptococci* population showed a little increase (from 3.08 to 3.1-3.2 log_{10}cfu/g) in CAP and PSVP samples while that of CVP and SAVP samples counts decreased (2.6-2.8 log_{10}cfu/g) by the end of storage.

Enterobacteriacea counts in fresh Pearlspot were 2.81 log_{10}cfu/g. By the end of storage, a 0.2 log increase was noticed in CVP, SAVP and PSVP samples whereas in CAP samples of pearlspot 0.4 log increase was observed. *Staphylococcus aureus* count was 1.2 log_{10}cfu/g in fresh pearlspot. On the day of sensory rejection, the counts decreased (0.2 log reduction) in all the samples. *Faecal streptococci* population was 2.58 log_{10}cfu/g in fresh Pearlspot. A 0.2-0.25 log reduction in *Faecal streptococcal* counts were noticed in CAP, CVP and SAVP samples. In PSVP pearlspot, 0.3 log reduction was found. The results of the study indicate good microbiological quality of fresh Pearlspot and Pearlspot samples stored at 0-2°C.

Clostridium botulinum toxin was not detected in any of the samples throughout the storage period. This indicates that there was no abuse of temperature during storage.

The microbiological data are in agreement with sensory data and TMA-N values. The low TMA-N values may be attributed to the low levels of *S. putrefaciens* by the end of storage of Pearlspot (<9%). In contrast, higher percentage of *S. putrefaciens* is found at the end of storage in Black pomfret contributing to high TMA-N values.

The treatments with sodium acetate and potassium sorbate influenced the microbial association of Black pomfret/Pearlspot stored under vacuum, but no pronounced inhibition was evident in aerobically developed microbiota. The significant changes in microbial attributes (compared to the air packed samples) due to preservative treatments can be summarized in three primary effects: (a) reduction of microbial load, (b) reduction of growth rates of fish spoilage organisms; and (c) in some cases increase in lag phases.

Research findings can be summarized as follows:

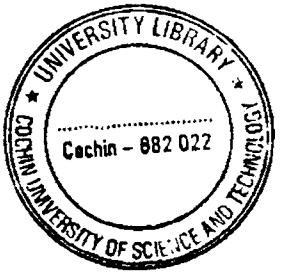
- 12μ polyester laminated with 300 gauge low density polyethylene was found to be suitable for vacuum packaging of fresh fish.
- Vacuum packaging alone, without preservatives is not of much use in extending the shelf life of Pearl spot and Black pomfret.
- 2% sodium acetate and 2% potassium sorbate treatment in conjunction with vacuum packaging can be successfully used for

preserving fish and thereby extending the shelf life of Pearlspot and Black pomfret with preservation of quality.

- The treatment with sodium acetate and potassium sorbate increased the shelf life of pearlspot stored under vacuum from 10 days to 15 days whereas the shelf life of air stored samples was only 8 days.
- The treatment with sodium acetate and potassium sorbate increased the shelf life of black pomfret stored under vacuum from 10 days to 16 days whereas the shelf life of air stored samples was only 8 days.
- *Pseudomonas* together with *S. putrefaciens* constituted major spoilers in both the species.
- Inhibitory effect of potassium sorbate on H_2S producing bacteria was found to be more significant than sodium acetate in both the species.
- Vacuum packaging can be safely applied to extend the shelf life of fresh fish, provided that proper refrigeration temperature (<38°F or 3.3°C) is absolutely maintained during distribution, storage, and retailing and in the home.

Today's opportunities and differences in the global market have also had their impact on the food processing, production, and consumption markets. International consumers demand the same quality, features, and products wherever they go. Vacuum packaging has the opportunities to establish a position in today's and tomorrow's dynamic markets of food processing, production and consumption. Vacuum technology can be applied to extend

storage life, lengthen distribution channels, decrease purchasing costs, preserve product quality, or even improve the product presentation.



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