Application of Active Packaging for Enhancing the Shelf life of Yellowfin Tuna (*Thunnus albacares* Bonnaterre, 1788) During Chilled Storage

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*I, Biji K B, hereby declare that the thesis entitled "**Application of Active Packaging for Enhancing the Shelf life of Yellowfin Tuna (Thunnus albacares Bonnaterre, 1788) During Chilled Storage**" is a genuine record of bonafide research carried out by me under the supervision of **Dr. C.N. Ravishankar,** Director, Central Institute of Fisheries Technology, Cochin and has not previously formed the basis of award of any degree, diploma, associateship, fellowship or any other titles of this or any other university or institution.*

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This is to certify that this thesis entitled "Application of Active Packaging for Enhancing the Shelf life of Yellowfin Tuna (*Thunnus albacares* Bonnaterre, 1788) During Chilled Storage" embodies the original work done by Mrs. Biji K.B, Reg No: 4080, under my guidance and supervision in the Fish Processing Division of Central Institute of Fisheries Technology, Cochin. 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.

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Biji K.B

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ABBREVIATIONS

AP	*Active packaging*
ADP	*Adenosine diphosphate*
AMP	*Adenosine monophosphate*
ATP	*Adenosine triphosphate*
AOCS	*American Oil Chemists' Society*
APHA	*American public health association*
ASTM	*American society for testing and materials*
AA	*Amino acid*
ANOVA	*Analysis of variance*
AOAC	*Association of Official Analytical Chemists*
atm	*Atmosphere*
BP	*Baired Parker*
BA	*Biogenic amine*
BAI	*Biogenic amine index*
BIS	*Bureau of Indian Standards*
IS	*Bureau of Indian standards*
CO_2	*Carbon dioxide*
CO2TR	*Carbon dioxide transmission rate*
cm	*Centimeter*
cfu	*Colony forming units*
CD	*Conjugated diene*
CAP	*Controlled atmosphere packaging*
CuSO4	*Copper sulphate*
MRS	*de Man, Rogosa and Sharpe*
DHA	*Docosahexaenoic acid*
EPA	*Eicosapentaenoic acid*
EMBA	*Eosin methylene blue agar*
EDTA	*Ethelene diamine tetra acetic acid*
EVOH	*Ethylene/vinyl alcohol*
EU	*European Union*
FAO	*Food and Agriculture Organization*
FFA	*Free fatty acid*
FFA	*Free fatty acid*
g	*Gram*
HDPE	*High density polyethylene*
HDPE	*High density polyethylene*
HMHDPE	*High Molecular High Density Polyethylene*
HPLC	*High performance liquid chromatography*
hrs	*Hours*

HCl	*Hydrochloric acid*
H_2O_2	*Hydrogen peroxide*
Hx	*Hypoxanthin*
HxR	*Inosine*
IMP	*Inosine monophosphate*
Fe	*Iron*
KFA	*Kenner faecal agar*
kg	*Kilogram*
LLDPE	*Linear low density polyethylene*
LDPE	*Low density polyethylene*
µl	*Microlitre*
mg	*Milligram*
ml	*Milliliter*
mm	*Millimeter*
min	*Minutes*
MAP	*Modified atmosphere packaging*
M	*Molar*
ln	*Natural logarithm*
N_2	*Nitrogen*
O_2	*Oxygen*
OTR	*Oxygen transmission rate*
ppm	*Parts per million*
PIA	*Peptone iron agar*
%	*Percentage*
PV	*Peroxide value*
PCA	*Plate count agar*
PP	*Poly propylene*
PVC	*Poly vinyl chloride*
PA	*Polyamide*
PEST	*Polyester*
PE	*Polyethylene*
PET	*Polyethylene terephthalate*
PS	*Polystyrene*
PUFA	*Polyunsaturated fatty acid*
PVDC	*Polyvinylidene chloride*
K_2SO_4	*Potassium sulfate*
pH	*Potential of hydrogen*
QIM	*Quality Index Method*
®	*Registered*
RH	*Relative humidity*

RPM	*Revolution per minute*
SFA	*Saturated fatty acid*
Σ	*Sigma*
NaOH	*Sodium hydroxide*
std	*Standard deviation*
SAS	*Statistical analysis software*
STAA	*Streptomycin thallus acetate acetidione agar*
T7	*Tergitol 7*
TPA	*Texture profile analysis*
TPA	*Texture profile analysis*
TBA	*Thiobarbituric acid*
TTI	*Time-temperature integrators*
TFS	*Tin free steel can*
TVB-N	*Total volatile base nitrogen*
™	*Trade mark*
TCA	*Trichloro-acetic acid*
TCA	*Trichloro-acetic acid*
TMA-N	*Tri-methyl amine-nitrogen*
UV	*Ultra violet*
UHT	*Ultra-heat treated*
UK	*United Kingdom*
USA	*United States of America*
VP	*Vacuum packaging*
VRBGA	*Violet Red Bile Glucose Agar*
H_2O	*Water*
WHC	*Water holding capacity*
WVTR	*Water vapour transmission rate*
WVTR	*Water vapour transmission rate*
ω-6	*Omega 6*
ω-3	*Omega 3*
^0C	*Degree celsius*
$Fe(OH)_2$	*Ferrous hydroxide*

Chapter -1
INTRODUCTION

Contents

1.1. Introduction

Seafood is a major source of high quality dietary protein and it is one of the rapidly growing components of modern diet. Fresh fish products are more perishable than any other food products due to their high water activity, neutral pH and presence of autolytic enzymes. The spoilage of fish and shellfish results from lipid oxidation, autolytic spoilage, and metabolic activities of microorganisms. The rate of spoilage is highly temperature dependent and can be controlled by using low temperature for storage. The degree of processing and preservation determines the storage life of fish. Packaging plays a key role in limiting any loss of fish quality.

Packaging has a significant role in the food supply chain and plays an integral role from production to consumption. It protects food from environmental conditions, such as light, oxygen, moisture, microbes, mechanical stress, and dust. It also ensures adequate labeling for providing information to customer and proper convenience to the consumer. Society is becoming increasingly complex and consumer is demanding for innovative packaging systems that are more advanced and creative than the traditional packaging systems.

The current trend in food industry is the development of mildly preserved wholesome and ready to eat natural convenience foods. Due to globalization, international markets are being extended resulting a wide distribution distances. These changes have encouraged the development of a wide range of active and intelligent food packaging systems in recent years. The potential for an active packaging technology to be successful for a product would depend on ability of the technology to control and inhibit the shelf life deteriorating spoilage reactions in the product. Adding value to the raw material will drive increased seafood consumption. Value addition is not only in gutting and cleaning and but in striving for ready to eat or ready to heat and eat form. These innovations will have to be supported by packaging that incorporates convenience. Active packaging has great potential in seafood industry. Active packaging is intended to change the condition of the packaged product to extend the shelf life or to improve sensory properties while maintaining the quality and freshness of the packaged food. To aid this, the packaging should absorb food deteriorating substance or should release substances such as preservatives, flavoring agents, antioxidants, antimicrobial agents, colours etc. that help in extending shelf life. Most important active packaging concepts include O_2 scavengers, ethylene scavengers, CO_2 scavengers and emitters, flavor absorbing and releasing systems, antimicrobial agents, antioxidant release systems etc. In many food products including seafood, CO_2 emitters along with O_2 scavengers can be used as a cost effective alternative to modified atmosphere packing (MAP) and vacuum packing (VP).

In India, active and intelligent packaging is still in a nascent stage and the market for active and intelligent packaging systems are expected to have a promising future by their integration into packaging materials or

systems. Regions such as North America and Europe dominate the active packaging market. Asia pacific and rest of the word are nascent markets for active packaging materials. Technological advancements in this field are expected to make these materials affordable, which would increase their demand in Asia Pacific and rest of the world countries (Transparancy market research, 2016). The Global active, smart and intelligent packaging market is supposed to grow at a compound annual growth rate (CAGR) of around 11.7% over the next decade to reach approximately $ 57billion by 2025 (Businesswire, 2016). There is an increase in demand for active and intelligent packaging because of the changing life style and the necessity of manufacturers to create longer shelflife goods to meet the demand from the public. In the year 2011, the market was dominated by controlled packaging. Active packaging was next in market share with nearly $8.8 billion in sales, and is expected to grow up to $11.9 billion by 2017 (BCC Research, 2013). Oxygen scavengers and moisture absorbers are the most commercially important sub categories of active packaging (Day, 2008). In 2012, gas scavengers were the leading active packaging product type in USA (Market Research, 2014) and the demand of gas scavengers will rise at a faster pace as a result of extended applications of oxygen scavengers in beverages and muscel foods (Businesswire, 2016). According to Freedonia Group Inc., gas scavenger demand will climb at a fast rate due to expanded applications for oxygen scavengers. Rapid growth from a low base is anticipated for antimicrobial packaging, encouraged by technological developments. However, cost and performance factors will still be a limitation (Market Research, 2014).

The need to reduce food waste and to optimize the use of raw materials would favour the implementation of packaging technologies such as

active and intelligent packaging. These technologies are designed to satisfy the increasing demand for food safety and longer shelf life, to contribute to a better stock management, and to favour brand differentiation. Therefore active and intelligent packaging is to support higher food quality, reduced waste and complaints from traders and consumers, and improved overall efficiency. These are the main reasons why novel packaging techniques like active and intelligent packaging systems are expected to play a key role in perishable food sectors such as seafood industry.

Tuna is one of the largest, most specialized and commercially important fish belonging to the genus *Thunnus* of the family Scombridae. They are found in tropical and temperate oceans around the world and account for a major proportion of the world fishery products. Tunas are unique among fishes since they possess body temperature several degrees higher than the ambient water. They have high metabolic rates that enable them to exhibit extraordinary growth patterns. Tunas are fast swimmers and are capable of traveling more than 48 km/h (Collette & Nauen, 1983). They are migratory and have few predators. Tunas are in great demand throughout the world market due to their excellent meat quality (Chang & Liu, 1995; FAO, 1997). Among tuna, yellowfin tuna (*Thunnus albacares*) are preferred more due to their better meat quality, yield of edible flesh and hence valued higher than skipjack tuna. The market for yellowfin tuna products is global and the three major countries consuming yellowfin tuna are Japan, the USA and the EU. The Japanese consume yellowfin tuna predominantly as sashimi. The EU and the USA markets consume the majority of their tuna products in an ambient format. In recent years the EU and the USA have experienced significant growth in the fresh tuna market. In this context, there is a need to adopt latest innovations like active and intelligent packing systems for

extending the shelf life and quality of tuna products. Studies on the use of active and intelligent packing are very limited in seafood and information are not available on the effect of oxygen absorber and carbon dioxide emitters on the shelf life extension of yellowfin tuna.

1.2. Hence the present study was undertaken with the following objectives:

- To develop an active packaging system with dual functionality for oxygen scavenging and carbon dioxide emitting into the package using various chemical mixtures
- To study the quality and nutritional value of yellowfin tuna
- To compare the shelf life and quality of yellowfin tuna packed under control air, vacuum and reduced oxygen atmosphere conditions during chilled storage (0-2^{0}C)
- To compare the developed active packaging system with commercially available oxygen scavengers for changes in physical, chemical and microbiological quality attributes of yellowfin tuna during chilled storage (0-2^{0}C)

Chapter -2
REVIEW OF LITERATURE

Contents

2.1 Introduction

Consumers prefer processed foods that are more easy to handle, store, and prepare. Modern consumers insist that such products also possess high quality, nutrition, and health benefits (Dey, 2000). The changes in consumer lifestyles have resulted in increased demand for two distinct types of seafood products. The first type includes fresh, chilled products that are conveniently processed, packed, and ready-to-cook. The second group consists of ready-to-eat seafood products, such as canned, fried and pickled products. In both types, the focus is the need for convenience and ease of handling. These market demands lead to development of novel techniques to extend the shelf life and add convenience to seafood (Venugopal, 2006). Maintenance of the quality of these fish and fishery products are more difficult than in the case of other muscle foods. Unlike all other food supplies, the production and process of seafood cannot be directly controlled,

or accurately predicted. There is an unusual diversity in the seafood industry depending on the types of harvest, fishing techniques, types of product, location of catch, etc. In addition, the characteristic nature of seafood makes them more vulnerable to food borne hazards (Bremner et al., 2002).

2.2Nutritional benefits of fish

Fish and fishery products play a major role in human nutrition. Studies shows that consuming fish at least 1-2 times per week has a positive effect on health (Sveinsdóttir et al., 2009; Thorsdottir et al., 2004). It is a good source of high quality protein containing all the essential amino acids in right proportion including sulphur containing amino acids methionine and cysteine (Lakshmanan, 2012). Fish protein is of high biological value since it contain all the essential amino acids. Cereal grains are said to be of low biological value since they are low in methionine, lysine, and tryptophan. Hence, a diet based on cereals with fish supplementation can increase the biological value significantly (Clucas & Ward, 1996). Unlike other terrestrial mammalian meat, fish is highly digestible due to the low stroma protein content and most species shows a protein digestibility greater than 90% (Lakshmanan, 2012). In addition, fish protein enable weight loss in overweight young adults and decrease undesirable types of blood fat with high antioxidation activity (Thorsdottir et al., 2007).

Fish oil contain high proportion of polyunsaturated fatty acids (PUFA) and is a rich source of essential fatty acids Ecosapentaenoic acid (EPA, S20:5 n-3) and Docosahexaenoic acid (DHA, C22:6 n-3). About 50% of the fatty acid in lean fish and 25% in fatty fish are polyunsaturated fatty acids (Mathew, 2010). On a unit caloric basis, fish provide a broad range of nutrients. A wide range of health benefits are found in PUFA. It has been

shown to have curative and preventive effects on inflammatory and cardiovascular diseases and has beneficial effects on hypertension, diabetes, macular degeneration and has anti cancer properties. It plays an important role in neurodevelopment of infants and glycemic control (Karmali et al., 1984; Nettleton, 1995; Rambjor et al., 1996; Conner, 1997; Mozaffarian et al., 2005; Caponio et al., 2011). It provides a number of nutritional advantages and therapeutic benefits on coronary heart disease, iron deficiency, protein deficiency, osteoporosis, arthritis, skin diseases, and defects in eyesight. Fish is also a rich source of vitamins and minerals. (Sherif, 2003; Mehmet, 2008)

2.3Quality and safety of seafood

The quality of a fishery product depends on two main factors, intrinsic and extrinsic. The intrinsic factors include species, sex, size, composition, spawning, presence of parasites, toxins, contamination with pollutants, and cultivation conditions (Love, 1988; Connell, 1995; Venugopal, 2006). The extrinsic factors influencing the quality of fish include location of catch, season, and methods of catch, on board handling, processing and storage conditions. One of the important characteristic of fish is its high perishable nature. Hence, the objective of processing and preservation of seafood is to prevent the undesirable changes in the nutritive and sensory characteristics of the products and to enhance the shelf life. According to Daun (1993) shelf life is defined as the maximal period of time during which the pre determined quality characteristics of food are retained. Waterman (1982) defined shelf life as being the same as storage life, keeping quality, keeping time and storage period. The definition given was length of time that a fish or fish product of initial high quality can be kept under

specified storage conditions before it becomes either significantly poorer in quality or unsuitable for consumption or sale .

The freshness of fish can be explained as an objective attribute, which must show normal odour, flavor, appearance, and texture characteristics of fish species to be used for samples (Olafsdottir et al., 1997). Freshness is an important factor determining the overall quality of seafood. The quality of fish can be estimated by sensory evaluation, microbial testing, measuring volatile compounds, rancidity tests, adenosine triphosphate (ATP) breakdown products and the physical changes in fish (Abbas et al., 2008). Trimethylamine (TMA) content (Tozawa et al., 1971), total volatile bases (TVB) (Antonacopoulos & Vyncke, 1989), individual nucleotides (Hattula et al., 1993; Jacober & Rand, 1982) and nucleotide ratios (K, Ki, H and G-values) are considered as indices of deterioration of fish quality (Burns et al., 1985; Ehira & Uchiyama, 1987; Karube et al., 1984; Luong et al., 1992). Levels of biogenic amines can also be useful in estimating freshness or degree of spoilage of fish since their development is associated with bacterial spoilage (Mackie et al., 1993).

2.4Postmortem Changes in fish

Live fish muscle is relaxed and elastic. Soon after death, blood circulation stops and it causes the aerobic respiration to cease. The end product of anaerobic respiration is lactic acid which results in a drop of pH in fish muscle tissue. The final post mortem pH depends on the amount of carbohydrate content in the muscle tissue. In the case of bivalve and molluscs, the carbohydrate content is comparatively higher than most of the teleost fish and crustaceans. During rigor stage, degradation of ATP leads to the irreversible interaction of actin and myosin to form actomyosin

(Venugopal, 2006). As the storage time proceeds, resolution of rigor occurs due to the proteolysis of proteins by cathepsins released from lysosomes resulting in tenderization of meat (Ravishankar, 2003). The other endogenous proteolytic enzymes causing softening of fish muscle are collagenase, serine protease, and calpains (Hultmann & Rustad, 2004; Yang et al., 2015). Rigor mortis influences the quality of fish fillets during processing. Fillets prepared during rigor will be stiff with low yield and will contract and shorten during the rigor stage causing gapping. Ideal stage for fish filleting is post rigor period (Ward & Baj, 1988; Connell, 1995).

The autolytic action of proteolytic enzymes subsequently leads to the degradation of ATP to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR) and hypoxanthin (Hx). The correlation between the nucleotide catabolism and freshness was well explained by Ashie et al. (1996) and Botta (1994). The freshness of fish can be explained in terms of K value. The K value is defined as the ratio of the sum of inosine and hypoxanthine concentrations to the total concentration of adenosine triphosphate metabolites (Abbas et al., 2008). Fish muscle contains several proteases that act on fish muscle during postmortem causing the deterioration of fish flesh. The autolytic enzymes in seafood include cathepsins, chymotrypsin, trypsin, and peptidases (Botta, 1994; Yongswawatdigul & Park, 2002). The differences in fish species and temperature of normal habitat may contribute to the postmortem degradation of fish muscle and the action of protease provides favorable conditions for bacterial growth (Delbarre-Ladrat et al., 2006). Rough handling can damage cellular structures that result in the release of autolytic enzymes including proteases that result in the enhancement of spoilage. One of the most adverse

effects of autolytic spoilage is the belly bursting of pelagic species (Ashie et al., 1996).

Chemical changes such as auto oxidation or enzymatic hydrolysis of lipids results in off flavor and colour development. The post mortem biochemical changes are highly influenced by the handling and processing practices. The post mortem changes that affect the quality and safety of seafood are associated with all the protein and ATP degradation, lipid oxidation, drop of pH, formation of bacterial degradation end products like trimethyl amine (TMA-N), ammonia, and other low molecular weight volatile base compounds, changes in texture, water holding capacity etc. (Alasalvar et al., 2002). The further deterioration of seafood quality is by microbiological activity. The intestinal and surface bacteria from equipment and humans contaminate fish during handling and processing. The rate of deterioration of seafood quality is highly temperature dependent and can be inhibited by the use of low storage temperature (Lakshmanan, 2012).

2.5Chilled storage

Icing and ice storage is one of the oldest and most effective methods of fish preservation. Chilling is the process of cooling fish or fish products to a temperature approaching that of melting ice. The purpose of chilling is to extend the shelf life of fish. Fresh fish is an extremely perishable commodity and deteriorates very rapidly at normal temperatures. To slowdown the rate of spoilage, fish should be cooled immediately after catch. Traditionally fish have been chilled by using ice, refrigerated sea water or chilled sea water (Rey et al., 2012; Barros-Velázquez et al., 2008). During chilling, the temperature is reduced to that of melting ice, 0^0C/32^0F (Shawyer & Pizzali, 2003). However significant decline of sensory quality and

nutritional value has been detected during chilled storage due to microbial and biochemical degradation mechanisms (García-Soto et al., 2014). The effect of organic acid icing system on the microbiological quality of hake, megrim and angler fish species during chilled storage were studied by Rey et al. (2012). Flake ice made with organic acids (ascorbic, citric and lactic)(800 mg/kg) was found to be an effective mixture for fish preservation due to its antimicrobial effect. Use of citric acid and lactic acid in ice to enhance the quality of hake, and megrim was also studied by García-Soto (2014). Treatment with lactic acid was studied by Kim et al. (1995) for shelf life extension of fish fillets and coated fish (Gogus et al. 2006).

2.6Methods for evaluating fish freshness

Methods for evaluating fish freshness and quality are based on the measurements of post mortem changes associated with sensory quality, chemical and physical changes, and microbial growth. Various methods used for evaluating fish freshness are discussed in detail below.

2.6.1 K value

The ATP breakdown compounds have been used as an effective tool for the estimation of fish freshness and present a very good correlation with the storage time of fish (Mazorra-Manzano et al., 2000). During storage, fish muscle nucleotide degrades as a result of endogenous biochemical changes (Whittle et al., 1990) and the level of ATP breakdown compounds are influenced by fish species, fish muscle types, storage conditions, (dark and white muscle), stress during capture, handling, (Özogul et al., 2006a,b; Erikson et al., 1997; Huss, 1995). Post mortem degradation of ATP goes through the intermediate products ADP, AMP, IMP, HxR and Hx (Church, 1998). Most of the adenosine nucleotides disappear quickly because they

degrade into IMP within 1-3 days after fish capture in ice storage and the degradation continues till the formation of inosine and hypoxanthine. The degradation of ATP to IMP is recognized as due to endogenous enzymes, but further degradation to inosine and hypoxanthine is also been connected with the microbial growth. According to Gram & Huss (1996), the bacterial growth has a positive correlation with the Hx production and the rate of bacterial production of Hx is better than the production rate by autolytic activities. Hypoxanthine is associated with the bitter taste and off flavor while inosine monophosphate is desirable as a flavor component enhancer and is associated with the acceptability of fresh fish (Dalgaard, 2000; Gram & Huss, 2000). Other indicators like Ki, H, G and P values, derived from K value, are also used. The suitability of one indicator or another depends on the degradation pattern of these metabolites.

K value (%)=[(Hx+HxR)/(ATP +ADP+AMP+IMP+Hx+HxR)] X 100 (Saito et al., 1959)

Ki value (%) = [(Hx+HxR)/ (IMP +Hx+HxR)] X 100 (Karube et al., 1984)

H value (%) = [(Hx)/(IMP + Hx+HxR)] X 100 (Luong et al., 1992)

G value (%) = [(Hx+HxR)/(AMP+IMP+HxR0] X 100 (Burns et al., 1985)

Wide variations in the K value are reported among different species on the day of rejection. 80% K value was detected for vacuum packed sardine stored at 4^0C (Özogul et al., 2004), 80% for seer fish stored with oxygen scavenger during chilled storage (Mohan et al., 2009a), 39% for sea bream stored at 2 ± 2^0C (Alasalvar et al., 2001), 72% for salmon stored at 1^0C (Sallam, 2007) 83% for the adductor muscle of Japanese baking scallop (Wongso & Yamanaka, 1998), 68.5% for catarina scallop at chilled storage (Ocaño-

Higuera et al., 2006). K value less than 20% is considered as 'Sashimi' quality and values between 20-60% are considered under acceptable range. K value above 60% have been considered as rejection point (Okuma & Watanabe, 2002). According to Saito et al. (1959), fishery products with K value lower than 20% is very fresh, with less than 50% as moderately fresh and higher than 70% as not fresh

2.6.2 Total Volatile Nitrogen (TVB-N) and Trimethyl Amine (TMA)

TVB-N is a product of bacterial spoilage and endogenous enzyme action of amino acids in fish muscle (Whittle et al., 1990; García-Soto et al., 2014). TVB-N compounds include off flavouring compounds like ammonia, monomethyl amine, dimethyl amine, trimethyl amine, and other volatile bases. (Debevere & Boskou 1996; Mendes et al., 2011). Fish decomposition is mainly influenced by the action of spoilage bacteria and autolytic enzymes. TVB-N thus produced is used to assess the quality of seafood stored at refrigerated temperatures. TVB-N values were reported to increase progressively as spoilage process and the level of 30 mgN/100 g fish muscle as the highest acceptable level as suggested by Gökodlu et al. (1998). Li et al. (2013a) observed that TVB-N values increases linearly or curvilinearly during chilled storage of yellow croaker. Similar results were also obtained for red drum (Li et al., 2013b), ray fish (Ocaño-Higuera et al., 2011) European eel (Özogul et al., 2005) sardine (Özogul et al., 2004).

In marine fish, TMA is produced by the decomposition of trimethyl amine oxide (TMAO) mainly due to the bacterial and enzymatic activity and hence can be related to the microbial deterioration and the production of spoilage substances (Ruíz-Capillas & Moral, 2005). TMA in marine fish is responsible for the characteristic fishy odour in spoiled fish

(Connell, 1995). Fish muscle is composed of low collagen, low lipid and high levels of soluble non-protein nitrogen (NPN) compounds. Trimethylamine oxide is present in all marine fishes can be broken down to TMA by endogenous enzymes. During chilled storage period, TMA is produced by the bacterial enzyme trimethyl amine oxidase. When the oxygen level is depleted, the spoilage bacteria can utilize TMAO as a terminal hydrogen accepter, thus allowing them to grow under anoxic conditions. Other low molecular weight sulphur containing compounds like H_2S, CH_3SH volatile fatty acids and ammonia are also produced during microbial spoilage (Sivertsvik, 2000a,b). TMA production in many fish species is also paralleled by bacterial production of Hx. Some of the spoilage bacteria such as *Shewanella putrefaciens* and *Vibrio* spp. also produce off smelling volatile sulfur compounds such as H_2S methyl mercaptan, and dimethyl sulfide, from sulfur containing amino acids. Mendes et al. (2011) observed a low rate of TMA production in soluble gas solubilisation (SGS) pretreated vacuum packed octopus samples. The reduction in TMA may be due to the reduction in growth of aerobic gram negative bacteria such as *S. putrifaciens* including TMA producing microorganisms. No legal limits are defined for TMA by EU. Connell, (1995) suggested a level of 12 mg TMA/100 g as a general limit for specific fish species.

2.6.3 Lipid Oxidation

Lipid oxidation is associated with early postmortem changes in the fish tissue. The main reason for the spoilage in fatty fish is the oxidation of lipids leads to the reduction of shelf life by the changes in taste, colour, odour, texture and the reduction of nutritional quality (de Abreu et al., 2011a, b). Lipid oxidation is comparatively more during frozen storage than during chilling (Huss, 1995). The lipid oxidation can be catalyzed by iron, heme

protein and lipoxygenase (Maqsood & Benjakul, 2011; Thiansilakul et al., 2010; Tokur & Korkmaz, 2007; Richards & Hultin, 2002). Two main types of lipid oxidation occur in foods systems are enzymatic and non-enzymatic. Enzymes such as peroxidase, lipoxygenase and microsomal enzymes from animal tissues can also initiate lipid peroxidation producing hydro peroxides. Breakdown of hydro peroxides into aldehydes, ketones, and alcohols causes development of off flavors and off odours. Fish lipids, rich in n-3 PUFA, are very susceptible to oxidation, giving rise to n-3 aldehydes that cause distinctive oxidative off-flavors. Fish lipids are also prone to hydrolysis by lipases with the formation of free fatty acids. Production of free fatty acids (FFA) is used to study the progress of lipid hydrolysis. A decrease of phospholipid followed by an increase in free fatty acid content during storage indicates the enzymatic hydrolysis of phospholipids (Koizumi et al., 1990). FFA is formed from triglycerides either by chemical or enzymatic hydrolysis process (Barthet et al., 2008). Lipid hydrolysis is found more in ungutted fish than in gutted fish, probably due to the involvement of lipases present in the digestive enzymes. Cellular phospholipases are known to hydrolyze the lipids, particularly, phospholipids that leads to increased oxidation of the hydrolyzed lipids (Huss, 1995). The enzymatic oxidation occurs mainly at the site of the oil water interface where the active site of enzyme moves towards the fat droplet. The oxidation caused by endogenous enzymes are influenced by both internal (enzyme content and composition) and external (intensity, feeding habits, temperature, season, etc) factors (Huss, 1995).

The non-enzymatic oxidation is induced by the presence of oxygen (Brockerhoff, 1974). When the fish is live, two types of antioxidant systems manage the oxidative damage suffered by macromolecules. One is the enzyme systems that eliminate the reactive oxygen species like hydrogen

peroxide, lipid hydro peroxide, superoxide dismutase (SOD), catalase, peroxidase etc. The second group of antioxidant compounds is low molecular weight compounds soluble in polar and apolar solvents and reacts with free radicles to form reactive compounds (de Abreu et al 2011b). The peroxide value (PV) is used to measure the degree of lipid oxidation and it indicates the amount of oxidized substances in the product. Oxidation of unsaturated fatty acids takes place in the presence of pro-oxidants like heme proteins and produce hydroperoxides (Undeland et al., 1998; de Abreu et al., 2011b). The hydro peroxides are the primary products of auto oxidation, its further oxidation leads to the production of a wide range of carbonyl compounds, furans, hydrocarbons, and it causes the rancid flavour to the product (Yanishlieva & Marinova, 2001). An increase of PV can be observed during the early stages of oxidation, due to the formation of hydro peroxides at a higher rate than the rate of decomposition. The value reaches a maximum and then decrease because of the lower availability of substrate and the instability of peroxide molecules, which results in lower rate of formation of PV with respect to the rate of decomposition (de Abreu et al., 2011a).

Fat oxidation also produces toxic compounds (Hansen et al., 2004; Indergard et al., 2014). The secondary oxidation of muscle tissue would produce malonaldehyde and it is a secondary decomposition product from polyunsaturated fatty acids and widely used as an indicator for the assessment of lipid oxidation (Connell, 1995). The malonaldehyde production depends on the amount of oxygen in the package. Presence of micro organisms, probable antioxidative enzymes, as well as the CO_2 dissolved in the tissue favour auto oxidation of polyunsaturated fatty acids (Goulas & Kontominas, 2007a; Ruíz-Capillas & Moral, 2001a; Masniyom et al., 2002; Bak et al., 1999). The malonaldehyde produced interacts with other

compounds like nucleosides, amines, proteins, amino acids, phospholipids or other aldehydes that are end products of lipid oxidation to form polymers which in turn decrease the quality of fish (Fernandez et al., 1997; Pournis et al., 2005; Nazemroaya et al., 2009; Sarma et al., 2000). The malonaldehyde produced can be measured by reacting it with 2-thiobarbituric acid in an acid solution. The bright pink colour thus formed can be measured at 538nm (Wang & Xiong, 2005). Low TBA values were also reported in some species during storage such as sea bream (Kyrana et al., 1997), European sea bass (Kyrana & Lougovois, 2002). According to Aubourg (1993), TBA values alone cannot indicate the actual degree of spoilage since malonaldehyde can interact with other compounds including nucleic acid, proteins, nucliosides, amines, phospholipids and such interactions vary significantly in different fish species.

The anisidine value is another indicator used for the determination of secondary oxidation products. It provides the aldehydes produced by the oxidation of unsaturated fatty acids and anisidine value is considered as an indicator of the oxidative history of the fat and oil present in fish (O'Sullivan et al., 2005). Conjugated diene (CD) and conjugated triene hydroperoxides are also produced during the initial stages of fat oxidation. They are relatively unstable like peroxides and decrease during advanced stages of rancidity (Aubourg et al., 2005a; Cho et al., 1989).

2.6.4 pH

The initial decrease in pH during storage may be due to the formation of lactic acid by the anaerobic glycolysis and the liberation of inorganic phosphate by the degradation of ATP (Liu et al., 2013; Delbarre-Ladrat et al., 2006). The bacterial fermentation of carbohydrates results in the

formation of organic acids that also cause the reduction of pH during the initial days of storage. Increased pH in fish muscle towards the end of shelf life indicates the accumulation of alkaline compounds including ammonia, dimethyl amine, TMA etc in fish muscle produced by microbial or endogenous enzymes (Li et al., 2013a,b; Khan et al., 2005; Cai et al., 2014). These alkaline compounds are mainly derived from microorganisms (Hebard et al., 1982). The pH changes in fish may differ depending on species, catching method, pre-slaughter stress, season, nutritional status etc. The rate and extent of pH change are important in determining quality of seafood. If the changes are less than or equal to 0.1 units, it is considered to be of first garde quality, if it is in the range of 0.1-0.2 units, it is referred as acceptable quality. Those with higher than 0.2 units suggests deterioration (Mazorra-Manzano et al 2000). In the case of tuna, studies indicate that pH ranges from 6.03 to 5.54 depends on the storage conditions (Lopez-Galvez et al., 1995; Ruíz-Capillas & Moral, 2005; Muela et al., 2014). pH above 7 may limit the shelf life of some fish species (Ruíz-Capillas & moral, 2001b).

2.6.5 Texture

Texture is one of the important characteristic that affects the overall quality and sensory acceptability of seafood. Gradual decrease of textural quality occurs during storage and becomes mushy during refrigerated storage (Nunak & Schleining, 2011). It also affects the mechanical processing of fillets in seafood industry. The deterioration of fish texture during storage is related to various intrinsic and extrinsic factors. Drip loss and fish muscle destruction may affect the texture of fish muscle (Delbarre-Ladrat et al., 2006; Huff-Lonergan & Lonergan, 2005).

Several studies are carried out to investigate the changes in texture during storage of seafood. Nunak & Schleining (2011) studied the changes in the texture of raw white shrimp stored in ice by instrumental texture analysis. The penetration test on the flesh using a spherical probe at a speed of 0.1mm/s using penetration work as a parameter gave the best result to clearly indicate the changes in textural properties of iced shrimp. Kagawa et al. (2002) used compression, tensile strength and penetration tests to evaluate the textural changes of squid during storage. Pornrat et al. (2007) used knife blade cutting test to determine the textural changes in prawn during storage. Espe et al. (2004) used Warner-Bratzler blade to evaluate the salmon texture.

2.6.6 Colour of flesh

Colour is one of the most important quality attribute used for evaluating the freshness of seafood at the point of sale. The colour of fish muscle depends on the pigment concentration, muscle structure characteristics, lipid oxidation etc (Gines et al., 2004). Myoglobin has been known to be the major contributor of colour of fish muscle depending on its derivatives, concentration and its stability (Chanthai et al., 1998; Postnikova et al., 1999; Chaijan et al., 2004). Therefore during handling and storage, a number of biochemical and microbiological changes occurs causing the reaction of myoglobin with other muscle components leading to discolouration (Faustman et al., 1992; O'Grady et al., 2001). Discolouration of tuna meat during storage were extensively studied and is caused by the formation of methemoglobin and metmyoglobin, the oxidized analogues of haemoglobin and myoglobin respectively. This process is influenced by many factors such as temperature, pH, and oxygen consumption reaction (Renerre & Labas, 1987). The lipid oxidation products can accelerate the

oxidation of myoglobin. According to Yin & Faustman (1994), the lipid oxidation and myoglobin oxidation in muscle foods occurs simultaneously and it boost each other.

Colourimetry measures the colours of fish samples with quantitative physical methods and are expressed using standard Hunter L*,a*, b* system. L* denotes lightness of the product on a scale from 0-100 ie from black to white. a* denotes (+) red or (-) green and b* denotes (+) yellow or (-) blue (Sanchez-Zapata et al., 2011). Dowlati et al. (2012) evaluated fish freshness using through colour changes of eyes and gills of farmed and wild caught sea bream. The results showed that a* parameter of fish eyes did not show clear trend with storage time. L*, b* and total colour difference of fish gills increased with storage time, but a* and c* (Chroma) decreased. The colour parameters of fish eye can be used as a low cost and easy method for fast online evaluation of fish freshness in food industry.

2.6.7 Water holding capacity (WHC) and Drip loss

Water holding capacity is a useful tool to describe the profitability and quality of fish muscle during storage because it affects the weight change during transportation and storage. Water holding capacity of muscle is defined as the ability of fresh meat to retain its own water during cutting, grinding, heating and pressing and during transportation, storage and cooking (Pearce et al., 2011; Hamm, 1986). WHC affects the drip loss during thawing, juciness, hardness, weight loss and shrinkage during cooking (Huff-Lonergan & Lonergan, 2005; Duun & Rustad, 2007; Shaviklo et al., 2010; Kaale et al., 2014) and it can influence the willingness to purchase the product.

Structurally drip loss is influenced by:

(1) Extent of lateral and transverse shrinkage of myofibrils at rigor
(2) The permeability of cell membrane to water
(3) The development of drip channels and extracellular space
(4) Post mortem cytoskeleton protein degradation (Offer et al., 1989; Huff-Lonergan & Lonergan, 2005; Bertram et al., 2004; Kristensen & Purslow, 2001; Hughes et al., 2014).

The drip contains water soluble proteins and amino acids which cause loss of quality (Cai et al., 2014) and nutritional compounds including minerals provide a favourable medium for bacterial growth. According to Duun & Rustad (2008), majority of drip comes from the immobilized water in the myofibrillar proteins in the muscle and it is mainly associated with the structure of muscle and muscle cells, fibre shrinkage, denaturation and degradation of proteins, rigor state of muscle, protein solubility, cell damage etc (Liu et al., 2013; Huff-Lonergan & Lonergan, 2005; Einen et al., 2002). Approximately 85% of the water in a muscle cell is held in myofibrils (den Hertog-Meischke et al., 1997; Huff-Lonergan & Lonergan, 2005). He et al. (2014) used a rapid and nondestructive method for the determination of drip loss and pH distribution in salmon fillets using near infra red (Vis-NIR) hyperspectral imaging.

2.6.8 Sensory evaluation

Seafood quality can be assessed by sensory methods based on the changes in apperaence, colour, odour, flavor and texture (Huidobro et al., 2000). Sensory liking is one of the strongest component for fish consumption intension and are simple, fast, sensitive and objective, but depends on the human judgment (Strachan & Nicholson, 1992; Simeonidou et al., 1998).

Fish consumers may have their own opinion for each products but usually find very difficult to differentiate in detail why they prefer one product to another. However, a descriptive sensory analysis with trained panels can provide unbiased, accurate and detailed description of each sensory attribute of the product (Sveinsdóttir et al., 2002).

The Quality Index Method (QIM) is a descriptive, fast and simple method developed by Tasmanian Food Research Unit (TFRU) to evaluate the freshness of seafood (Huidobro et al 2000). It is a freshness grading system based on important sensory parameters of raw fish and a score system from 0 to 3 demerit points (Sveinsdóttir et al., 2002; Barbosa & Vaz-Pires, 2004; Sant'Ana, 2011). It evaluates the sensory parameters and attributes with most significant changes in each species during spoilage. Higher points are given as storage time progresses (Erkan & Özden, 2007; Huidobro et al., 2000). If there occurs a linear correlation between quality index (QI) and storage time, the total demerit scores may be used to predict the remaining shelf life (Botta, 1994). QIM is an important method for predicting the end of shelf life with the support of other evaluation methods. QIM scheme for whole black spot and seabream using sensory analysis, Torry meter measurements and bacterial counts of specific spoilage organisms during chilled storage was developed by Sant'Ana et al. (2011).

Preference mapping are one of the recent method used to study the consumer preference of various food products. Preference mapping studies were carried out in various products like meat (Helgesen et al., 1997), beverages (Geel et al., 2005; Guinard et al., 2001), fruits (Thybo et al., 2003; Daillant-Spinnler et al., 1996), cheese (Westad et al., 2004), cod products (Sveinsdóttir et al., 2009). According to Oliveira et al. (2015), individual disagreements may occur sometimes beyond the sensory assessment due to

some biological factors. The food texture is the result of different natural stimuli and its assessment is a complex process that indicates visual perception of the products, their response to handling and the integration of the senses experienced in the mouth during chewing and swallowing (Costell & Durán 2005). According to Serfert et al. (2010) an intense and specific training and performance monitoring is required for sensory panel to gain knowledge about the ability and limitations of the sensory panel and to achieve accurate evaluation of samples.

2.6.9 Biogenic amines (BA)

The most important biogenic amines; histamine, tyramine, tryptamine, putrescine and cadaverine, are formed from free amino acids, namely histidine, tyrosine, tryptophan, ornithine and lysine, respectively. Spermidine and spermine arise from putrescine. Putrescene is the precursor of ornithine. The conentration of free amino acids especially histidine is important since histidine is precursor in the biosynthesis of histamine (Biji et al., 2016). The biogenic amines are produced through decarboxylation of specific free amino acids by exogenous decarboxylase enzymes released by microorganisms assoiated with seafood (Lee et al 2015). Among the biogenic amines, histamine is potentially hazardous and the causative agent of histamine intoxication associated with the consumption of seafood (Morrow et al., 1991; Biji et al., 2016). Cadaverin and putrescine have been reported to enhance the toxicity of histamine (Taylor, 1985) through interfering with histamine detoxification system. It inhibits the intestinal histamine metabolizing enzymes, diamine oxidase and histamine N-methyl transferase (Stratton et al., 1991)

Biogenic polyamines, such as putrescine, cadaverine, spermidine, spermine and agmatine, are potential carcinogens they can be converted to nitrosamine when exposed to nitrite (Bills et al., 1973). Common symptoms of BA intoxication are migraine, brain hemorrhage, heart failure, hypertension, urticaria, headache, flushing, abdominal cramps, hypertension, and hypotension (Rice et al., 1976).

The association of type of fish and biogenic amine poisoning may reflect the amount of consumption of a specific fish. A maximum histamine content of 50 mg/kg (FDA, 2011), 200 mg/kg (EC, 1991) and 300 mg/kg has been established in the European Community for acceptance of tuna and other fish belonging to the Scombridae and Scombresocidae families. The maximum tyramine level recommended (in food) to be in the range of 100-800 mg/kg (Brink et al., 1990; Rabie et al., 2009). Ienistea (1973) reported the deleterious effects in relation to the amount of histamine ingested at one meal as mild poisoning (8-40 mg histamine), disorders of moderate intensity (70-1000 mg histamine), severe incidents (1500-4000 mg Histamine)

Table 2.1 Histamine toxicity level (EFSA, 2011)

Histamine concentration	Fish quality	Health effects	Reference
<50 mg/kg	normal	safe for consumption	Bartholomew et al., 1987
50-200 mg/kg	mishandled	possibly toxic	Bartholomew et al., 1987
200-1000 mg/kg	unsatisfactory	probably toxic	Bartholomew et al., 1987
≥500 mg/kg	not reported	Toxic	Dalgaard et al., 2008
>1000 mg/kg	unsafe	Toxic	Bartholomew et al., 1987

2.6.9.1 Conditions supporting the formation of biogenic amines

2.6.9.1.1 ***Substrate availability***: The availability of substrate amino acids is one of the prerequisites for biogenic amines synthesis. Proteolysis is a crucial factor, because it is directly related to the availability of free amino acids that provide a substrate for biogenic amine formation (EFSA, 2011).

Table 2.2 Regulatory limits of Histamine in seafood (Biji et al., 2016)

Country	Limit	Reference
EU	Fishery products from fish species associated with high amount of histidine n = 9, c = 2, m= 100mg/kg, M = 200mg/kg Fishery products which have undergone enzyme maturation treatment in brine n = 9, c = 2, m = 200 mg/kg, M = 400mg/ kg	Commission regulation (EC) No 2073/2005
USFDA	50 ppm (50 mg/kg)	FDA, 2011
Australia, Germany and New Zeland Food Standards Code (FSC)	200mg/kg	Ezzat et al. (2015)
South Africa and Italy	100 mg/kg	Ezzat et al. (2015)

N= number of units comprising the sample, c = number of sample units giving values over m or between m and M

2.6.9.1.2 Microorganisms producing biogenic amines

Many different bacterial species of the Enterobacteriaceae family possess histidine decarboxylase activity and produce histamine. The species include *Morganella morganii, Klebsiella pneumonia, Hafnia alvei, Proteus vulgaris, P mirabilis, Enterobacter aerogenes, E cloacae, Serratia fonticola, Raoultella ornithinolytica, R. planticola, Providencia stuartii, Citrobacter freundii* (Kim et al., 2003). In addition to the enteric bacteria, *Vibrio*

alginolyticus, Clostridium spp., Acinetobacter lowffi, Plesiomonas shigelloids, Pseudomonas putida, P fluorescens, Aeromonas spp., and *Photobacterium spp*. have also been reported as histamine formers (Biji et al., 2016; Chen et al., 2010; Lopez-Sabater et al., 1994; Middlebrooks et al., 1988; Okuzumi et al., 1994; Ryser et al., 1984; Yatsunami & Echigo, 1991).

2.6.9.1.3 *Storage temperature*

Storage temperature is one of the most important factor contributing to biogenic amines formation. High amount of biogenic amines were produced under high storage temperature and are reported by many authors (Du et al., 2002; Rodtong et al., 2005; Kim et al., 2002; Wei et al., 1990; Chong et al., 2011). Kim et al. (2002) observed that 25^0C is optimum for histamine production by *Morganella morganaii* in artificially contaminated muscles of mackerel, albacore, and mahi-mahi. The level of histamine was 4610 ppm for mackerel after 24 hours of storage; 3430 ppm for albacore; and 3340 ppm for mahi-mahi. The effect of temperature abuse on histamine formation in tuna muscle stored at different temperature was studied by Economou et al. (2007) who found that icing temperature retarded histamine formation. Du et al. (2002) observed 18 ppm of histamine in tuna after 9 days storage at 0^0C, whereas 68.8 ppm, 564 ppm and 4500 ppm histamine in yellowfin tuna stored at 4°C, 10°C and 22^0C respectively.

The most effective way to prevent scombroid fish poisoning is by proper refrigeration of fish from the point of production to the final end user. Once the enzyme histidine decarboxylase is present in fish, it can produce histamine in fish even though the bacteria are not active. The enzyme can be activated at or near refrigeration temperatures (Lehane Olley, 2000). Histamine cannot be destroyed by freezing or heating such as normal

cooking, hot smoking or canning (Arnold & Brown, 1978; Taylor, 1986; Lehane & Olley, 2000; Flick et al., 2001; FDA/CFSAN, 2001; Kim et al., 2003; Hungerford, 2010), hence it can be used as food quality indicators to identify those who use poor quality raw material for processing (Sim et al., 1992). Thermal processing eliminates all potential bacteria responsible for histamine formation. However applying heat after histamine formation in the product will not ensure food safety (Naila et al., 2010)

2.6.9.2 Control of biogenic ammines

The biogenic amine formation is highly temperature dependent and its production can be reduced by lowering temperature through inhibition of microbial growth and the reduction of enzyme activity (Du et al., 2002). The inclusion of carbon dioxide in Modified atmosphere packs may inhibit the growth and increase the lag phase of microorganism with amino acid decarboxylase activity. The effect of MAP on the inhibition of biogenic amines in fish was studied by Özogul et al., 2002a,b; Emborg et al., 2005; Özogul & Özogul, 2006). Emborg et al. (2005) studied the effect of vacuum packaging and modified atmospheric packaging on biogenic amine formation at 2^0C and 10^0C in tuna muscle inoculated with psychrotolerant bacteria and observed that histamine achieved toxic level in chilled vacuum packaged tuna steaks at 2^0C. The effect of O_2 scavenger on the formation of biogenic amines during chilled storage of seer fish (*Scomberomorus commerson*) was carried out by Mohan et al. (2009b) and observed that biogenic amine content increased significantly in air packs compared to O_2 scavenger packs. The chitosan edible food coating along with MAP on histamine inhibition in Atlantic bonito fillet was studied by Alak et al. (2010). The fillets packed with chitosan film and modified atmospheric packaging showed significantly lower histamine concentration and enterobacteriaceae count.

2.7 Microbiology of fish and fishery products

Microbial activity is responsible for spoilage of fresh and lightly preserved seafood. The microorganisms associated with most seafood reflect the microbial population in their aquatic environment (Colby et al., 1993), species, feeding habits and the method of capture (Gram & Melchiorsen, 1996). Microorganisms are found on gills, skin and intestine of live and freshly caught fish. The total number of microorganisms varies with conditions and the normal range is between 10^2-10^7cfu/cm^2 on surface of skin and 10^3-10^9cfu/g on intestine and gills (Huss, 1995). Even though the fish muscle is sterile at the time of catch, they become quickly contaminated with bacteria present on skin, gills, intestine, equipments, human during handling and processing (Sivertsvik et al., 2002a,b). The microflora of temperate waters is dominated by psychrophilic, aerobic or facultative anaerobic gram negative rod shaped bacteria in particular *Pseudomonas*, *Shewanella*, *Vibrio*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Photobacterium* and *Aeromonas* (Lalitha & Surendran, 2006; Pantazi et al., 2008). The micro flora of tropical fish is dominated by gram positive and enteric bacteria (Lalitha & Surendran, 2006)

During chilled storage, there occurs a shift in bacterial types. The part of the micro flora which grows on the products is determined by the intrinsic and extrinsic parameters. The bacterial groups causing the important chemical changes during fish spoilage often consist of a single species, the specific spoilage organisms (SSO). For many fish stored under aerobic conditions in ice, *Shewanella putrefaciens* (Gram et al., 1987), *Pseudomonas* has been identified as the main single bacteria. *Shewanella putrefaciens* and *Psuedomonas* spp. have been recognized as the SSO in iced fresh fish such as cod (Dainty et al., 1983; Gram & Huss, 1996). Under anaerobic conditions,

like in MAP, VP and active packaging, the spoilage bacteria differ from aerobic spoilage. *Photobacterium phosphorum*, *Lactobacillus*, and *Carnobacterium* has been identified as the organisms responsible for spoilage in VP and MAP packs (Dalgaard, 1995; Dalgaard et al., 1993; Emborg et al., 2002; Gram & Huss, 1996).

Fish products with high salt contents may spoil due to the growth of halophilic bacteria, anaerobic bacteria, and yeast, whereas in lightly salted fish, spoilage could be due to lactic acid bacteria and certain *Enterobacteriacea* (Huss, 1995). The application of filtered smoke for the suppression of bacterial growth and oxidative stability were studied in various fish species like yellowfin tuna (Ludlow et al., 2004; Kristinsson et al., 2008) Spanish mackerel (Garner & Kristinsson., 2004), mahi mahi (Demir et al., 2004). Leydon et al., (2005) studied the effect of filtered smoke on the suppression of aerobic bacterial growth on thawed tilapia fillets.

2.8 The Protective role of packaging

According to Coles (2003) packaging is defined as a means of ensuring safe delivery to the ultimate consumer in sound condition at ideal cost. Traditional food packaging is meant for containment, protection and convenience (Paine, 1991; Robertson, 2006). The package is used to protect the product from the deteriorative effects of the external environmental conditionals like heat, light, presence or absence of moisture, pressure, microorganisms, and gaseous emissions etc. It also provide the consumer with the greater ease of use, time saving, convenience and contain product of various size and shapes (Yam et al., 2005; Marsh & Bugusu, 2007). The package also plays an important role in marketing and selling the product (Brennan & Brian, 2006; Yam et al., 2005) and it act as a silent sales man. It

also communicates important information about the product type, how to prepare and it communicates the nutritional information to the consumer (Gopal, 2007). The key safety objective of packaging materials which comes in contact with food is to be inactive as possible. While the novel packaging systems like active and intelligent packaging concepts are based on the active interaction between packaging environment and the food to provide protection to the food.

A wide range of materials are used for packing food including paper, paperboards, fibreboards, regenerated cellulose films, polymer films, semirigid and rigid containers made from polymer materials, metal foil, rigid metals, glass, timber, textiles and earthenware. Very often, a combination of two or more materials is employed to package one product. It is very important to select a suitable packaging material for packing food materials since success of most preservation methods depends on appropriate packaging (Gopal, 2007). Another function is to provide information about the product. It provides the legal requirements, product ingredients, use etc. to the consumers. Packaging also provides convenience for the pack handlers and users throughout the packaging chain, brand communication, promotion etc (Coles, 2003)

2.9 Factors affecting the selection of packaging material

It is important to select a packaging material of sufficient strength and rigidity to reduce damage due to compression loads. Metal, rigid plastic and glass materials are used for primary or consumer packages. Fibre board and timber materials are used for secondary or outer packages. The incorporation of cushioning materials into the packaging can protect against impacts, shock and vibration. Corrugated papers and boards, pulp board and foamed plastics are examples of such cushioning materials. Restricting

movement of the product within the package may also reduce damage. This can be achieved by shrink wrapping or tight wrapping. Thermoformed trays may be used to provide compartments for individual items such as eggs and fruits. Mechanical damage may occur as a result from sudden impacts or shocks during handling and transport, vibration during transport by road, rail and air, compression due to staking etc. Appropriate packing along with good handling and transportation are necessary to reduce the incidence of such mechanical damages (Gopal, 2007).

2.10 Overview of some of the developments in packaging

Innovations in packaging development started in 18th centuary itself with the invention of canning by Nicolas Appert in 1809. Many subsidiary packaing materials and equipments were developed subsequently. In 1810, Peter Durand designed the soldered tinplate canister and commercialized its use in thermal preservation of food (Davis, 1967). Paper bag machine was developed in 1852 by Francis Wolle of Pennsylvania, USA (Davis, 1967). In 1874, Oliver Long patented (No. 9948) the use of lined corrugated materials (Maltenfort, 1988). The first machine made folding carton was produced by Robert Gair of New York in 1879, (Davis, 1967). In 1884, Quaker Oats packaged the first cereal in a folding box (Hine, 1995). Crown cap for glass bottles was patented in Baltimore, USA by William Painter in 1892 (Opie, 1989). Paraffin wax coated paper milk containers were being sold by G.W. Maxwell in San Francisco and Los Angeles in 1906, (Robertson, 1993). Creams were packed in waxed paperboard cartons during 1910s. Regenerated cellulose film (RCF) was developed in 1912. In 1915, John Van Wormer of Toledo, Ohio, commercialized a folded blank paper bottle (Robertson, 2002). The use of frozen foods in retail packs using cartons with waxed paper wrappers was commercialized by Clarence

Birdseye who founded Birdseye Sea foods in 1923. Cellophane was also introduced by using cellulose casting process in 1920s (Coles, 2003). Imperial Chemical Industries (ICI) Ltd. Commercially produed polymerized ethylene (PE) in 1939. In 1946, polyvinylidene chloride (PVdC) was introduced as moisture barrier resin. Retortable pouches used were developed in 1950s for US army. The use of Aluminium trays for frozen foods, aluminium cans and squeezable plastic bottles was introduced in 1950s. Tetra packs were introduced in 1956 from low-density polyethylene extrusion coated paperboard. In 1960s, two-piece drawn and wall-ironed (DWI) can was developed. Tin free steel can (TFS) was developed in 1960s. In 1970s the bar coding system was introduced for retail packing in USA. Modified Atmosphere Packing was also introduced for retail packing. Frozen foods in microwaveable plastic containers, bag-in-box systems, and flexible packaging systems were also developed in 1970s. Co-extruded plastics for squeezable sauce bottles, microwavable retort plastic containers, PET-coated dual-ovenable paperboard for ready meals were developed in 1980s. In 1990s, digital printing of graphics on carton sleeves and labels for food packaging was first introduced in UK (Coles, 2003).

2.10.1 Vacuum packaging

In vacuum packing the product is placed inside the pack and air is evacuated and sealed. Vacuum packing helps to reduce the oxygen content inside the pack. It reduces the growth of aerobic spoilage microorganisms. It also offers excellent protection against desiccation and rancidity (Gopal, 2007). Anaerobic spoilage is associated with the vacuum packed sea foods. A serious problem associated with vacuum packed seafood is the threat of *Clostridium botulinum*.

2.10.2 Modified atmosphere packaging

Carbon dioxide is the active gas of MAP because it inhibits growth of many normal spoilage bacteria (Sivertsvik et al., 2002b; 2003). The effect of carbon dioxide on bacterial growth is complex and four activity mechanisms of CO_2 on microorganisms has been identified by Faber (1991) and Daniels et al. (1985). These include modification of membrane functions comprising the nutrient uptake and absorption, direct inhibition of enzymes or decrease in the rate of enzyme reaction, penetration of bacterial membranes leading to intracellular pH changes and direct changes in the physicochemical properties of proteins. Probably a combination of all these activities accounts for the bacteriostatic effect (Sivertsvik et al., 2002a).

The bacteriostatic or antibacterial effect of CO_2 relates to the pH effect on bacteria where CO_2 dissolves and creates an acidic environment within the cell. It leads to the intracellular accumulation of k^+. The pH drop not only affects the growth of microorganisms but also create a localized pH drop in the bacterial cells (Ryan & Ryan, 1972; Parkin & Brown, 1982).

MAP is the removal or replacement of the atmosphere surrounding the product before sealing in high barrier materials (McMillin et al., 1999). MAP is used for prolonging the shelf life period of fresh or minimally processed foods. The shelf life of perishable products like meat, fish, fruits and vegetables can be prolonged with MAP since it slows the natural deterioration of the product. MAP with CO_2 as the active component have been reported to inhibit microbial growth in fish especially against gram negative microorganisms (Goulas & Kontominas, 2007b; Hovda et al., 2007; Rosnes et al., 2006; Sivertsvik, 2007; Stamatis & Arkoudelos 2007; Chen & Xiong, 2008). In controlled atmosphere packaging (CAP) continuous monitoring and control of the environment is maintained to a stable gas

atmosphere and other conditions such as temperature and humidity within the package (Brody, 1989).

MAP for meat requires a barrier of both moisture and gas permeation through packaging materials to maintain the desired head space gas composition during storage (Blakistone, 1999). Classically fresh red meats are stored in modified atmosphere packages containing 80% O_2:20% CO_2 (Georgala & Davidson, 1970) and cooked meats are stored in 70% N_2:30% CO_2 (Papkovsky et al., 2002).

2.10.2.1 History of MAP

The use of underground sealed silos for grain storage used at least 2000 years before was considered as the first modified atmosphere for food storage. In this case, atmosphere modification was detected as "foul air" that was dangerous to enter and was likely a result of O_2 depletion and CO_2 accumulation due to the respiratory activity of the grain. The modified atmosphere was unintentional, although probably beneficial. The foul air in the storages would presumably control rodent and insect pests, thereby acting to preserve the quality and storage life of the grain (Beaudry, 2007). Polyethylene, a polymer with very high O_2 and CO_2 permeability, and perhaps the most widely used polymer in MAP applications today, was first synthesized in an industrially practical way by Imperial Chemical Industries (ICI) in Britain in the mid to late 1930s (Beaudry, 2007).

2.10.2.2 Gases used in MAP

2.10.2.2.1 Carbon dioxide

The function of carbon dioxide in MAP is to inhibit growth of spoilage microbes (Seideman & Durland, 1984). Carbon dioxide (CO_2) is

soluble in both water and lipid it has a bacteriostatic and fungistatic properties. Carbon dioxide lowers the intra and extra cellular pH of tissue including that of microorganisms. It affects the membrane potential and influence the equilibrium of decarboxylating enzymes of microorganisms (Gopal, 2007). CO_2 increases the lag phase and a slower rate of growth of microbes during logarithmic phase (Brody, 1989). This bacteriostatic effect is influenced by the partial pressure of CO_2, concentration of CO_2, , volume of headspace gas, type of micro organism, age and load of initial bacterial population, microbial growth phase, growth medium used, storage temperature, acidity, water activity, and type of product being packaged (Church, 1994; Faber, 1991; Phillips, 1996; Daniels et al., 1985; Dixon & Kell 1989; Parkin & Brown, 1982). Pathogens like *Clostridium perfringens* and *Clostridium botulinum* are not affected by the presence of carbon dioxide and their growth is encouraged by anaerobic conditions. Carbon dioxide is most effective in foods where the normal spoilage organisms consist of aerobic, gram negative psychrotropic bacteria (Hotchkiss, 1989; Phillips, 1996).

The CO_2 is flushed into the modified atmosphere package by evacuating the air and flushing the appropriate gas mixture into the package prior to sealing. Another method to create a modified atmosphere for a product is either to generate the CO_2 and/or remove O_2 inside the package after packaging or to dissolve the CO_2 into the product prior to packaging. Both methods can give appropriate packages with smaller gas/product ratio to the package (Gopal, 2007).

The solubility of CO_2 decreases with increasing temperature, hence MAP products should be stored at lower temperatures, preferably at $<3^0C$ to get the maximum antimicrobial effect. Also the temperature

fluctuations will usually eliminate the beneficial effects of CO_2. The rate of absorption of CO_2 depends on the moisture and fat content of the product. If product absorbs excess CO_2, the total volume inside the package will be reduced, giving a vacuum package look known as "package collapse". Excess CO_2 absorption along with "package collapse" results in the reduction of water holding capacity and further drip loss to the products (Gopal, 2007).

2.10.2.2.2 Nitrogen

Nitrogen is used in MAP as an inert filler gas to reduce the proportions of the other gases and to maintain pack shape (Bell & Bourke, 1996). N_2 helps to prevent package collapse because of its low solubility in fat and water. Displacing O_2 in the pack can delay oxidative rancidity and prevent the growth of aerobic micro organisms. Nitrogen can also indirectly influence the micro organisms in perishable foods by retarding the growth of aerobic spoilage organisms (Faber, 1991; Phillips, 1996). The combination of gases used in MAP depends on many factors, such as the type of the product, packaging materials and storage temperature. The packaging system should have sufficient headspace to provide enough gas to interact with the food product (Parry, 1993).

2.10.2.2.3 Oxygen

The major function of oxygen is to maintain myoglobin into its oxygenated form, oxymyoglobin (Faustman & Cassens, 1990). Appearance, especially colour is an important quality characteristic influencing the consumer's decision to purchase. In fresh red meats, myoglobin exist in one of three chemical forms. Deoxymyoglobin, which is purple in colour and it rapidly oxygenated to cherry red oxymyoglobin on contact with air. Over time, oxymyoglobin is oxidised to form metmyoglobin which is brown in

colour. Hence metmyoglobin is associated with the brown discoloration in fresh red meat (Faustman & Cassens, 1990). Oxygen concentration inside the headspace favour oxidation of oxymyoglobin to metmyoglobin (Ledward, 1970). Hence, in order to minimise metmyoglobin formation in fresh red meats, oxygen must be removed from the packaging environment to below 0.05% or should be present at saturating levels (Faustman & Cassens, 1990). High oxygen concentration within MAP promote oxidation of muscle lipids over time with causing rancidity of fresh meat colour (O'Grady et al., 1998).

2.10.2.2.4 Carbon monoxide

Carbon monoxide is colourless, tasteless and odourless gas that is highly reactive. It is less soluble in water. The commercial application is limited because of its toxicity and the formation of explosive mixtures with air (Mullan & McDowell, 2003).

2.10.3 Active and intelligent packaging

The key safety objective for traditional packaging materials which comes in contact with food is to be inert as possible. While the innovative packaging systems like active and intelligent packaging concepts are based on the useful interaction between package headspace and the food to provide active protection to the food.

2.10.3.1 Active packaging

Packaging may be termed active when it performs some role other than providing an inert barrier to the external environment (Rooney,1995a,b). Active packaging can be defined as a system in which the product, package and the environment interact in a positive way to extend the shelf life or to achieve some characteristics (Miltz et al., 1995). It is also be defined as a type of packaging that changes the condition of the packaging to

extend shelf life or to improve safety or sensory properties while maintaining the quality of the packaged food (Ahvenainen, 2003). According to regulation EC/1935/2004 and EC/450/2009 active materials and articles are intended to extend the shelf life or to maintain or improve the condition of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or environment surrounding the food (Sivertsvik 2007; Floros et al., 1997). The goal of active packaging is to enhance the preservation of food in the package. This can be achieved by temperature control, oxygen removal, moisture control, addition of chemicals such as salt, sugar, carbon dioxide or natural acids or a combination of these with effective packaging (Robertson, 2006; Restuccia et al., 2010; Biji et al., 2015). These developments in active packaging have led to advances in many areas including delayed oxidation in muscle foods, controlled respiration rate in horticultural products, microbial growth and moisture migration in dried products. In addition, active packaging also manipulates the selectivity to modify the atmospheric concentration of gaseous compounds inside the package by coating, lamination, micro perforation, co-extrusion, or by polymer blending. (Brody et al., 2008).

Active packaging sometimes also referred as smart or interactive packaging as it is intended to sense the changes in external and internal environments and to respond by changing its own properties and hence the internal package environment (Brody et al., 2001). According to Day (2001, 2003) active packaging has been considered as a subset of smart packaging and referred to as the incorporation of certain additives into the packaging materials or within the packaging materials to maintain and extend the shelf life of the product. According to Wagner (1989), smart packaging can be

defined as doing more than just offer protection. They interact with the product, and in some cases, actually respond to changes.

2.10.3.1.1 History of active packaging

The use of tin plates for the construction of cans may be the first used active package in which tin is sacrificially corroded to protect the iron base can. It also helps to protect food from contamination of iron. The next development was the introduction of aluminium cans and further development was the introduction of sulfur staining resistant lacquers instead of tin. It prevented the decomposition of sulfur containing amino acid present in the food and the subsequent staining on the metal surface. Another development was the application of zinc oxide which mask the reaction and products making not observable in the white lacquer (Rooney, 1995a). Active packaging plays an additional role of protection of food to the classic purposes of any packaging, viz. containment, protection, convenience and communication. (Robertson, 1993).

2.10.3.1.2 Global market of active controlled and intelligent packaging

Over the past decade, active and intelligent packaging have experienced a tremendous growth and change as new products and technologies have challenged the status quo of the traditional forms of food and beverage packaging (Kotler & Keller, 2006). Active packaging firstly introduced in the market of Japan in 1970s and it slowly raised the attention of the food industry in Europe and USA in 1990s (Restuccia et al., 2010). The global market for food and beverages of active and intelligent coupled with controlled/modified atmosphere packaging (CAP/MAP) increased from $15.5 billion in 2005 to $16.9 billion by the end of 2008. The demand for packaged ready to eat food has witnessed a significant surge in demand of

active and intelligent packing systems. The global active, smart and intelligent packaging market is expected to grow at a compound annual growth rate (CAGR) of around 11.7% to reach approximately $57billion by 2025 (Globenewswire, 2016) The largest market for active and intelligent packing is forcasted to be the US with a CAGR of 7.4% worth $3.6 billion by the end of 2021. Japan will be the second largest market with a predicted worth of $2.36 billion in the next decade (Bakeryandsnacks, 2016).

2.10.3.1.3 Active packaging systems

The best known and most widely used active packaging technologies for food today are those engineered to remove undesirable substances from the head space of a package through absorption, adsorption, or scavenging.

2.10.3.1.3.1 Oxygen scavengers

The most widely used active packaging technology for foods today are oxygen scavengers. The presence of oxygen in a package accelerates the oxidative deterioration of food. Oxygen facilitates the growth of aerobic microbes, off flavor and odour development, colour changes and nutritional losses and overall shelf life stability of muscle foods (Day, 2001, 2008; Rooney, 1995a, 2005; Hogan & Kerry, 2008). Therefore control of oxygen levels in food packages is important to limit the rate of such spoilage reactions in food. Even though the oxygen sensitive foods can be packed in modified atmosphere packaging (MAP) or vacuum packaging, but it does not remove oxygen completely. Oxygen which permeates through the packaging film cannot be removed through the system. By the use of oxygen scavengers, which absorbs the residual oxygen after packaging, quality

changes in oxygen sensitive foods can be minimized (Vermeiren et al., 1999; Kerry et al., 2006).

According to Brody (2001) oxygen scavengers are defined as the materials employed in the process of removing oxygen or preventing it from entering in package environment of food products subject to undesirable oxidative reactions. The oxygen absorbers are designed to reduce oxygen levels to less than 100ppm in package headspace. Relatively inexpensive oxygen scavengers are used to remove the residual oxygen remaining in the MAP (Day, 2003, 2008; Robertson, 2006). Oxygen scavengers can be hot melt bonded to the inner wall of the package. An alternative to sachets include package inserts in the form of cards, sheets or layers coated onto the inner walls of the package (Rooney, 1995b). Incorporation of oxygen scavenger into the package eliminates the risk of accidental rupture of the sachets and inadvertent consumption of their contents (Suppakul et al., 2003). Oxygen scavenging compounds can be dispersed or blended with high permeability films like polyethylene, which allow rapid diffusion of oxygen and water from the headspace or from food to the reactive ingredients (Day, 2008)

Removal of oxygen from package interiors improves shelf life by sub-optimizing the environment for aerobic microbial growth and for adverse oxidative reactions such as rancidity. Ferrous iron based oxygen scavengers depends on the moisture content of the product for its activation. Oxygen scavengers are designed to reduce the oxygen levels to less than 100ppm in the package head space. Iron based scavengers are based on oxidation of powdered iron forming nontoxic iron oxide (Ashie et al., 1996). Oxygen absorbers are used to create oxygen free conditions in head space. Oxygen absorbers in fish cakes, fish pudding and mackerel fillets showed only

marginal effect on the microbial growth. However a significant effect on the O_2 absorber was observed in packages with salmon fillets. It inhibited the development of rancidity in both mackerel and salmon fillets (Sivertsvik, 1997).

The commercially available oxygen scavengers utilize one or more of the following technologies: iron powder oxidation, ascorbic acid oxidation, photosensitive dye oxidation, enzyme oxidation, saturated fatty acid oxidation, immobilized yeast on solid material etc. (Floros et al., 1997; Vermeiren et al., 1999).

Majority of currently available oxygen scavengers are based on iron powder oxidation in the form of small sachets containing various iron based powders containing as assortment of catalysts. The chemical substances react with the water supplied by the food to produce a reactive hydrated metallic reducing agent that scavenges oxygen within the food package (Day, 2008).

$$Fe \rightarrow Fe^{2+} + 2\ e^-$$

$$1/2O_2 + H_2O + 2e^- \rightarrow 2OH^-$$

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$$

$$Fe(OH)_2 + 1/4O_2 + 1/2H_2O \rightarrow Fe(OH)_3$$

The sachets are designed to reduce O_2 levels to less than 0.01%. A rule of thumb is that 1g of iron will react with 300 cc of O_2 (Labuza, 1987). When the initial O_2 concentration at the time of packaging and the O_2 permeability of the packaging material is known, an absorber can be selected with a higher capacity than the needed calculated capacity. In that way, total absence of O_2 is guaranteed during storage life of the product (Vermeiren, 1999). The effects of iron based O_2 absorber on the quality and safety of

freshwater cat fish (*Pangasius sutchi*) and seer fish (*Scomberomorus commerson*) during chilled storage was studied by Mohan et al. (2008, 2009 a, b)

Some O_2 scavengers use an enzyme reactor surface that would react with some substrate to scavenge incoming O_2. Glucose oxidase, which is an oxidoreductase that transfers two hydrogen from -CHOH group of glucose to O_2 with the formation of glucano-delta-lactone and hydrogen peroxide (H_2O_2). The lactone then spontaneously reacts with water to form gluconic acid (Labuza et al., 1989).

The reaction is:

$$2G + 2O_2 + 2H_2O \rightarrow 2GO + 2H_2O_2$$

Where G is the substrate.

Since H_2O_2 is an objectionable end product, catalase is introduced to break down the peroxide (Brody & Budny, 1995)

$$2H_2O_2 + \text{catalase} \rightarrow 2H_2O + O_2$$

Coupled enzyme systems are very sensitive to changes in pH, water activity, salt content, temperature and various other factors. Since they require water, it therefore, cannot be effectively used for low water foodstuffs (Graff, 1994). One application for glucose oxidase is the elimination of O_2 from bottled beer or wine. The enzymes can either be part of the packaging structure or put in an independent sachet. Both polypropylene (PP) and polyethylene (PE) are good substrates for immobilizing enzymes (Labuza & Breene, 1989).

Labuza & Breene (1989) suggested that a film with bound enzyme which would effectively scavenge O_2 would be in an acceptable price range. A 1998 patent (Strobel & Gagnon, 1998) describes a polyolerin

structure containing glucose oxidase. Glucose oxidase, other enzymes are used for O_2 scavenging, including ethanol oxidase which oxidises ethanol to acetaldehyde (Labuza & Breene, 1989). Oxygen scavengers can be directly incorporated into the packaging structure itself. Low molecular weight ingredients may be dissolved or dispersed in a plastic or the plastic may be made from a polymeric scavenger. Oxyguard of Toyo Seikan Kaisha, Japan is an iron based absorber which can be incorporated into a laminate (Vermeiren et al., 1999; Rooney, 1995a). The main disadvantage of O_2 scavenging films are the capacity and speed of O_2 scavenging films are considerably lower than the iron based O_2 scavenger sachets (Day, 1998).

Ascorbic acid is another O_2 scavenging component which can be used for removing oxygen from packages. Ascorbic acid gets oxidized to form dehydroascorbic acid and sulphite to sulphate. This is used in barrier packaging such as crown caps, plastic or metal closures. Crown caps are mainly used in beer to protect it from oxidation of flavours (Darex technical information, 1998). Another O_2 scavenging technique involves sealing of a small coil of an ethyl cellulose film containing a dissolved photosensitive dye and a singlet O_2 acceptor in the headspace of a transparent package. Due to illumination of the film with light of the appropriate wave length, excited dye molecules sensitize O_2 molecules, which have diffused into the polymer, to the singlet state. These singlet O_2 molecules react with acceptor molecules and are thereby consumed (Rooney, 1985). The photo chemical process involved can be presented as follows

$$\text{Photon} + \text{dye} \rightarrow \text{Dye}^*$$
$$\text{Dye}^* + O_2 \rightarrow \text{Dye} + O_2^*$$
$$O_2^* + \text{Acceptor} \rightarrow \text{Acceptor oxide}$$
$$O_2^* \rightarrow O_2$$

The use of an O_2 scavenger can influence different food properties. O_2 scavengers are effective in preventing growth of moulds and aerobic bacteria. But O_2 free atmosphere can favour the growth of anaerobic pathogens such as *Clostridium botulinum* (Labuza, 1987). O_2 absorbers prevent the oxidation of oils and fats to prevent rancidity. It helps to prevent the discolouration of pigments of both plants and animals. It also helps to prevent the loss of taste and nutritive elements (Schozen et al., 1997; Berenzon & Saguv, 1998; Gill & Mc Ginnes, 1995). O_2 scavengers can be used alone or in combination with MAP. Oxygen scavengers eliminate the need for MAP machinery and can increase packaging speeds. However, it is usually more common in commercial applications to remove most of the atmospheric O_2 by MAP and then use a relatively small and inexpensive scavenger to remove the residual O_2 within the food package (Day, 1998). For an O_2 absorber to be effective, the packaging material needs to have high O_2 barrier property, otherwise the scavenger will rapidly become saturated and lose its ability to trap O_2 (Smith et al., 1990; Rooney, 1995b).

Packaging problems involving the need for oxygen scavenging may be divided into two classes based on the origin of the oxygen that needs to be removed. The headspace and dissolved oxygen present at the time of closing most packages of foods and beverages. Removal of some or all of this oxygen is required at a rate greater than that of the various food degradation processes that occur in the food. In this case, a headspace scavenger package is required. The oxygen that enters the package by permeation or leakage after closing needs to be removed, preferably before contacting the food. The scavenger required is a chemically enhanced barrier (Day, 2003)

Table 2.3 Physical and chemical principles applied in active packaging (Rooney, 1995a)

Principle	Application
Porosity Control	Gas pressure release Gas composition balance
Polymer Permeability	Gas composition balance Temperature compensation
Melting of Waxes	Time-Temperature indicators Temperature compensation
Energy Shielding	Microwave shielding Thermal insulation Shock absorption
Energy Transfer	Microwave crisping UV absorption
Inorganic-Organic Oxidation	Oxygen scavenging Oxygen permeation barrier Oxygen indicator Carbon dioxide generation Ethylene scavenging Taint removal
Enzyme Catalysis	Oxygen scavenging Time-Temperature indication Lactose removal Cholesterol removal
Acid-Base Reaction	CO_2 absorption CO_2 generation Odour absorption
Adsorption	Taint removal Oxygen scavenging Ethylene scavenging Water removal
Absorption	Humidity buffering Condensation control Drip collection
Hydrolysis	Sulfur dioxide release
Desorption	Ethanol release Water release
Organic Reactions	Ethylene removal Oxygen barrier

Yeast as oxygen scavenger

Yeast is used to remove oxygen from the headspace of hermetically sealed packages (Brody, 2001). Immobilized yeast is applied into the liner of a bottle closure (Edens et al., 1992). Yeast is also used in a pouch within the package (Nezat & Jerry, 1985). Yeast present in the package when moistened, the yeast is activated and respires, consuming oxygen and producing carbon dioxide plus alcohol. In the bottle closure application, any carbon dioxide and alcohol produced would enter the contents (Brody et al., 2001).

Shortcomings of oxygen scavenger sachets (Brody et al 2001)

- Risk of accidental consumption of oxygen scavenging agent
- Sachets cannot be made into very small size
- Containers with narrow opening face the problem of inserting the scavengers into the container
- The oxygen scavenger powder in the bag tends to coagulate into a lumpy shape and has a reduced surface area for contacting air. Therefore, in order to produce the scavenging of oxygen at a desired rate, it is necessary to provide a larger quantity of oxygen scavenging powder than with the theoretical oxygen scavenging capacity.

Table 2.4 Some currently known active packaging systems and their applications (Floros et al, 1997; Brody et al., 2002)

Active Packaging system	Substances used	Applications
O_2 scavenger	Powdered iron oxide, Ferrous Carbonate, Photosensitive dye oxidation, Ascorbic acid oxidation etc.	Fresh and dry fish, sausages, smoked and cured fish
CO_2 emitter	Ascorbic acid, Ferrous Carbonate, metal halide etc.	Fresh fish
Moisture regulator	Silica gel, Polyvinyl alcohol etc.	Fresh and dry fish products
Ethanol emitter	Encapsulated Ethanol	Fresh and semi dry fish products
Antimicrobial packing	Sorbates, Benzoates, Ethanol, Ozone, Antibiotics etc.	Fresh fish and value added fish products
Antioxidant release	BHA, BHT, Ascorbic acid, Tocopherol etc.	Fresh, dried and smoked fish, fish oil
Flavour absorbing	Baking soda, activated charcoal	Fresh and dry fish
Flavour releasing	Many food flavours	Fresh and heat processed fish products
Colour containing	Various food colours	Surimi, smoked and red meat fish, shrimps
Light absorbing and regulating	UV blocking agents	Dry fish and fish oil
Microwave susceptors	Metalized thermoplastics	Ready to eat fish meals
Insect repellant	Low toxicity fumigants (pyrethrins, permethrin)	Dried, smoked and fried fish

2.10.3.1.3.2 Carbon dioxide absorbers and emitters

Carbon dioxide can be added to the packaging environment to suppress the microbial growth in certain products such as fresh meat, poultry, fish, cheese, and backed goods (Lopez-Rubio et al., 2004) and to reduce the respiration rate of fresh produce (Labuza, 1996). Therefore, high CO_2 levels (10-80%) are desirable for such food items to extend the shelf life (Labusa, 1996). High levels of CO_2 inhibit the surface microbial growth and thereby extend shelf life (Labuza, 1996; Coma, 2008). Aerobic bacteria like

Pseudomonas are inhibited by moderate to high levels of CO_2 (10-20%), microorganisms like lactic acid bacteria can be stimulated by CO_2. Food pathogens like *Clostridium perfringens, Clostridium botulinum* and *L. monocytogenes* are minimally affected by CO_2 levels lower than 50% (Yingyuad et al., 2006; Kerry et al., 2006; Vermeiren et al., 1999) The method of dissolving sufficient amount of CO_2 into the product in one to two hour in pure CO_2 prior to retail is called as soluble gas stabilization (SGS) (Sivertsvik, 2000a). SGS is not an active packaging technology by definition, but application of active releasing systems based on ferrous carbonate or ascorbic acid which simultaneously release CO_2 and scavenge O_2 is desirable (Rooney, 1995a; Kerry et al., 2006). The commercial CO_2 emitters usually contain ferrous carbonate and a metal halide catalyst absorbing O_2 and producing equal amount of CO_2. Carbon dioxide can be produced by allowing the exudates from the product to react with a mixture of citric acid and sodium carbonate inside the drip pad (Bjerkeng et al., 1995). Commercial manufacturers include Mitsubishi Gas Chemical Co Ltd (Ageless TM type G), and Multisorb Technologies Inc (FreshPax Type M)® USA. Standard MAP tray with perforated false bottom with sachet can be applied for muscle foods. The exudates from the food act with the sachet to release CO_2 to the package and prevent package collapse (Kerry et al., 2006).

Carbon dioxide scavengers are used to remove excess CO_2 in packages. CO_2 scavengers are mainly used in fresh roasted coffees which produce significant amount of CO_2 of hermetically sealed in packs directly after roasting, which leads to the bursting of package (Day, 2008). Mitsubishi Gas Chemical Company offers sachets specially for CO_2 scavenging. The use of CO_2 scavengers replaces the 'aging' process after coffee roasting and thereby prevents the loss of desirable coffee volatiles (Brody et al., 2001). A

mixture of calcium oxide and activated charcoal has been used in coffee pouches to remove CO_2. Calcium oxide are also used in containers for the shipment of fruits and vegetables to remove excess carbon dioxide produced during the respiration, and thus obviates the effects including reduced pH, colour and flavor changes (Kerry et al., 2006).

2.10.3.1.3.3 Antimicrobial packing

Antimicrobial packing is a form of active packaging in which the packaging acts to inhibit, reduce, or retard the growth of microorganisms that may be present in the packaged food or packaging material itself (Appendini & Hotchkiss, 2002). To control undesirable microorganisms in foods, antimicrobial substances can be incorporated in or coated onto food packaging materials (Labuza & Breene, 1989). Natural antimicrobial agents include extracts from spices like cinnamon, clove, thyme, rosemary, oregano and other plant extracts like onion, garlic, radish mustard and horseradish. Other natural antimicrobials are derived from substances produced from fungal and bacterial action like polypeptide nisin, natamycin, pediocin, and various bacteriocins (Nicholson, 1997). Antimicrobial package material can be classified into two types: those containing antimicrobial agents that migrate to the surface of the packaging material, and those are effective against surface microbes without migration of the active agent to the foods (Han, 2000). The direct surface application of antibacterial substances onto food have limited applications because the active substance are neutralized on contact or diffuse rapidly from the surface to the food. The incorporation of antimicrobial agent to meat formulations may result in partial inactivation of active compounds by meat constituents and therefore exert a limited effect on surface micro flora (Quintavalla & Vicini, 2002). Therefore if the

antimicrobial substance can be released from the package for an extended period from transportation, storage and distribution could be more efficient.

The antimicrobial food packaging material has to extend the lag phase and reduce the growth phase of microorganisms in order to extend the shelf life (Han, 2000). The major potential food applications for antimicrobial agents include meat, poultry, bread, cheese, fruits and vegetables (Labuza, 1987). Bioactive agents including antimicrobials are incorporated into polymers are used for drug and pesticide delivery, textiles, surgical implants and other biomedical devices. Silver substituted zeolite is the most common antimicrobial agent used in Japan. Silver ions inhibit a wide range of metabolic enzymes and it has a strong antimicrobial activity with a broad spectrum. The silver zeolite is laminated as a thin layer on the food contact surface of the laminate. Silver ions release from the laminate when the aqueous solution from the food enters the exposed cavities of the porous structure (Ishitani, 1995; Quintavalla & Vicini, 2002). Commercial examples of silver substituted zeolites include Zeomic®, Apacider®, AgIon, Bactekiller and Novaron. Antimicrobial enzymes like lactoperoxidase and Lactoferrin, antimicrobial peptides like megainins, cecropines, defensins, natural phenols like hydroquinons and catechins, fatty acid esters, antioxidant phenolic, antibiotics and metals like copper are incorporated into polymers (Hotchkiss, 1997; Appendini & Hotchkiss, 2002). For heat sensitive antimicrobials like enzymes, and volatile compounds, solvent compounding may be effective. Antimicrobial packaging materials must be in contact with the food if it is nonvolatile so that the antimicrobial agents can diffuse to the surface.

Packaging systems that release volatile antimicrobials include chlorine dioxide, sulphur dioxide, carbon dioxide and ethanol. In this system,

polymer need not be directly in contact with the food. Here the antimicrobial agents are directly incorporated into the polymer or into the carriers that may be extruded or coated into the packaging materials. The packaging material used for the volatile antimicrobial system should possess high barrier properties to prevent the loss through permeation (Appendini & Hotchkiss, 2002). Ethicap® and Antimold® are ethanol releasing sachets and they act by absorbing moisture and releasing ethanol vapour (Smith et al., 1995). Ethanol vapour generators are mainly used for high moisture bakery products, cheese and fish. Chlorine dioxide is another antimicrobial agent effective against bacteria, fungi and viruses. Chlorine dioxide can be generated by using sodium chlorite and acid precursors which are embedded in a hydrophobic and hydrophilic phase of a copolymer. Moisture from the food when come in contact with the hydrophobic phase, acid is released which reacts with the sodium chlorite releasing chlorine dioxide (Smith et al., 1995). Applications for this technology are just beginning and have a potential to reduce food safety risks for meat, poultry, fish, dairy, confectionary and baked goods.

Some antimicrobial packaging uses covalently immobilized antibiotics or fungicides to suppress the growth of microbes by immobilization of non food grade antimicrobial substance without diffusional mass transfer (Brody et al., 2002). Examples of antimicrobials with functional groups are peptides, enzymes, polyamines and organic acids. Functional groups in polymers commonly used for food packaging materials include ethylene vinyl acetate, ethylene methyl acetate, ethylene acrylic acid, polyvinyl chloride copolymer, polystyrene, etc. Some polymers like chitosan are inherently antimicrobial and are used in films and coatings. Chitosan has been used as a coating to protect fresh vegetables and fruits from fungal

attack. It also acts as a barrier between the nutrients contained in the product and microorganism (Cuq et al., 1995).

Antimicrobial edible coatings and films prepared from polysaccharides, proteins and lipids have variety of advantages like biodegradability, edibility, biocompatibility, aesthetic appearance, barrier properties etc. Whey protein coatings and films can incorporate adequate amounts of edible antimicrobial agents like lysozyme, nisin, potassium sorbate etc. (Gennadios et al., 1997; Lopez-Rubio et al., 2004). Ming et al. (1997) used pediocin or nisin fixed on cellulose casing to inhibit the growth of *Listeria monocytogenes* on ham, turkey meat and beef. Nisin and lysozyme in soy protein and corn zein films are used to inhibit *Lactobacillus plantarum* and *Escherichia coli* on laboratory media (Dawson et al., 1997; Padgett et al., 1998). Antimicrobial packaging can play an important role in reducing the risk microbial contamination to extend the shelf life of foods.

2.10.3.1.3.4 Moisture regulators

A major cause of food spoilage is the presence of moisture and the purpose of moisture regulator is to lower the water activity of the product to suppress the microbial growth (Vermeiren et al., 1999). Moisture also affects the gas and vapour permeability of hydrophilic plastic packing films (Rooney, 1995a). In the case of fresh fruits and vegetables, respiration followed by condensation occurs when one part of the package is cooler than the other areas. If not properly removed, this moisture will be absorbed by the product. Soluble nutrients leach into the water causing low consumer appeal and microbial spoilage. Moisture content in the pack causes softening of dry crispy products, caking of hygroscopic products like milk powder, instant coffee powder, sweets etc. (Vermeiren et al., 1999). Excessive moisture

evaporation through the packaging material also cause case hardening or desiccation on the surface of the product. Hence liquid water control or humidity buffering are used in moisture sensitive products to maintain the desired relative humidity in the package headspace.

Moisture absorbent pads, sheets and blankets are used for controlling liquid from foods like fish, meat, poultry, fruits and vegetables. Large sheets and blankets are used for absorbing melted ice during the air freight transportation of chilled fish (Day, 1998). Drip absorbent sheets basically consist of two layers of a micro porous polymer like polyethylene or polypropylene sandwiched with a super absorbent polymer in the form of free flowing granules. This sheet is sealed at the edges to allow water absorbent to be in place rather than aggregating towards one edge of the sheet when tilted (Rooney, 2005). Thermarite® Pvt Ltd (Australia), Toppan™ (Japan), Peaksorb® (Australia), Luquasorb™ (Germany), Fresh-R-Pax™(Atlanta) are some of the commercial moisture absorbent sheets, blankets and trays. Desiccants are mainly used in products like cheese, chips, nuts, candies, spices etc. Desiccants like silica gel, molecular sieves, calcium oxide are used for dry foods while micro porous bags or pads of inorganic salts and protected layer of solid polymeric humectant are used to buffering the humidity inside the cartons (Day, 1998; Brody et al., 2001). Commercial examples of sachets include MINIPAX® (USA), STRIPPAX® (USA), Desipak® (USA), Tri-Sorb® (USA), 2-in-1™ (USA), and the moisture absorbing labels include DesiMax® (USA) (Vermeiren et al., 1999).

2.10.3.1.3.5 Antioxidant release

Antioxidants are widely used in food to improve the oxidation stability of the food to prolong the shelf life. Antioxidants are incorporated in packaging films as a source of antioxidants in some foods since the increased

consumer demand for reduced antioxidants and additives in foods. Antioxidant incorporation can also stabilize the polymer inorder to protect the films from degradation (Smith et al., 1990; Rooney, 1995b). The effect of Butylated Hydroxy Toluene (BHT) incorporated HDPE packs for oat flake was studied by Han et al. (1987). The outward migration of BHT was 70% and 25% of the BHT was found in the cereal. The outward loss can be prevented by using an extra polymer layer with low permeability (Miltz et al., 1988). But the effect of BHT on human health has heen questioned due to accumulative effect of BHT in human adipose tissue (Wessling et al., 1998). Incorporating natural antioxidants like Vitamin C, and E on packaging films can reduce oxidative reactions like development of rancid odour and colour changes in fatty fishes. Vitamin E is also safe and effective for low to medium water activity cereal and snack food products (Labusa & Breene, 1989; Day, 2003) and proved to be stable under processing conditions with excellent solubility in polyole fins (Wessling et al., 1998; Vermeiren et al., 1999).

2.10.3.1.3.6 Ethylene scavengers

Ethylene is a natural plant growth hormone which accelerates respiration of fruits and vegetables, induces fruit ripening, fruit softening and senescence even at low concentration (Abeles et al., 1992). It causes yellowing of vegetables, russet spotting on lettuce and has detrimental impact on shelf life of many fruits and vegetables (Zagory, 1995). Much research effort has been undertaken to remove ethylene from the fresh produce packaging and storage areas. Controlled atmospheric storage with low oxygen and high carbon dioxide concentrations are practiced to reduce the respiration rate.

Potassium permanganate immobilized on inert minerals are available in sachets for packages and blankets that can be placed in product holding rooms without integrating into the food contact packaging material (Labuza & Breene, 1989; Day, 2003). Potassium permanganate oxidizes ethylene to acetate and ethanol. During the process, colour changes from purple to brown indicating the remaining C_2H_4 scavenging capacity. Products based on $KMnO_4$ cannot be integrated into food contact materials, because KMnO4 is toxic and has a purple colour. Typically, such products contain 4 to 6% $KMnO_4$ on an inert substrate with a large surface area such as perlite, alumina or silica gel, (Zagory, 1995).

Activated carbon base with various metal catalysts also removes ethylene effectively. Activated charcoal impregnated with palladium catalyst is also used to scavenge ethylene from fresh produce. SedoMate® (Japan), Neupalon^{TM} (Japan), Hatofresh® (Japan) is some of the commercial sachets based on activated carbon capable of scavenging ethylene (Rooney, 2005; Takashi, 1990). Neupalon^{TM} sachets are capable of scavenging both ethylene and moisture from the surroundings (Rooney, 1995b; Labuza & Breene, 1989; Day, 2003). Nissho, a Japanese company incorporates finely ground coral of pore size 10-50 µm on polythene sheets to absorb ethylene gas (Brody et al., 2001). Use of 1-methylcyclopropane (1-MCP) is another alternative to minimize the effect of ethylene. It binds to the ethylene receptors in plant tissue and prevents the hormonal action of ethylene. (Blankenship & Dole, 2003). Shelf life of many fruits, vegetables and flowers can be extended by the application of 1-MCP.

2.10.3.1.3.7 Flavor or odour absorbers and releasers

The volatile compounds that accumulate inside the package as a result of food degradation such as aldehydes, amines and sulfides can be

selectively scavenged. Sometimes it masks the natural spoilage reactions and misleads consumers about the condition of the product inside the package (Day, 2008). Flavor scavengers prevent the cross contamination of pungent odour while transportation of mixed loads. Odour proof packages were developed for the transportation of Durian fruit (Morris, 1999). The package consist of an odour impermeable plastic like polyethylene terephthalate (PET) or polyethylene of suitable thickness together with a port to allow for the passage of respiratory gases and a sachet made from a mixture of charcoal and nickel to absorb odour. Volatile amines formed due to the protein breakdown in fish muscle can be removed by incorporating acidic compounds like citric acid in polymers (Hoshino & Osanai, 1986). Flavor scalping of polyethylene was studied by Sajilata et al. (2007). This is due to the lipolytic nature of polyethylene, it attracts large amount of non polar compounds such as volatile flavours and aroma in foods. Use of high barrier packaging materials can also prevent the absorption of other nonfood odours like taints (Brody et al., 2008). Flavor release act as a means to mask the off odours coming from the food. It is also used to release desirable flavours into the food and to encapsulate pleasant aromas that are released upon opening the package to influence the sensory acceptability of the product (Nielson, 1997)

2.10.3.1.3.8 Edible coatings and films

Edible coating films can be eaten together with the food material. Edible coating films are nowadays used in the preparation of many seafood products. Methyl cellulose and hydroxypropyl cellulose are used to reduce the uptake of fat during frying. Alginates are used to reduce the moisture loss from fresh fish, while palmitates are used to reduce moisture loss from frozen

fish. Other edible films include whey protein isolates, coconut oil, and acetylated mono and diglycerides (Brody et al., 2001).

Table 2.5. Commercially available active packaging systems (Biji et al 2015)

Trade Name	Manufacturer	Principle	Type
Ageless	Mitsubishi Gas Chemical Co Ltd, Japan	Iron Based	Oxygen Scavenger
Freshilizer	Toppan Printing Co Ltd, Japan	Iron Based	Oxygen Scavenger
Freshmax, Freshpax, Fresh Pack	Multisorb Technologies, USA	Iron Based	Oxygen Scavenger
Oxyguard	Toyo Seikan Kaisha Ltd, Japan	Iron Based	Oxygen Scavenger
$Zero_2$	Food Science Australia, Australia	Photosensitive Dye	Oxygen Scavenger
Bioka	Bioka Ltd, Finland	Enzyme Based	Oxygen Scavenger
Dri-Loc®	Sealed Air Corporation, USA	Absorbent Pad	Moisture Absorber
Tenderpac®	SEALPAC, Germany	Dual Compartment System	Moisture Absorber
Biomaster®	Addmaster Limited, USA	Silver Based	Antimicrobial Packing
Agion®	Life Materials Technology Limited, USA	Silver Based	Antimicrobial Packing
SANICO®	Laboratories STANDA,	Antifungal Coating	Interleavers
Neupalon	Sekisui Jushi Ltd, Japan	Activated Carbon	Ethylene Scavenger
Peakfresh	Peakfresh Products Ltd, Australia	Activated Clay	Ethylene Scavenger
Evert-Fresh	Evert-Fresh Corporation, USA	Ativated Zeolites	Ethylene Scavenger

The chitosan coating of fresh fillets of cod (*Gadus morhua*) and herring (*Clupea harengus*), sardine (*Sardinella longiceps*) reduced moisture losses, lipid oxidation, headspace volatiles (total volatile basic nitrogen, TMA, and hypoxanthine), and growth of microorganisms as compared to uncoated samples. The preservative efficacy and the viscosity of chitosan

were interrelated. Thus, chitosan can be used as an edible coating could enhance the quality of seafoods during storage (Jeon et al., 2002; Mohan et al., 2012). Skinless tilapia (*Dreochromis niloticus*) fillets were covered with a gelatin coating containing benzoic acid as an antimicrobial agent. After seven days of storage under refrigeration, tilapia fillets coated with gelatin containing benzoic acid had acceptable TVBN contents, moderate microbial loads, and showed no significant sensory difference from fresh fillets. The results indicate that an antimicrobial gelatin coating is suitable for preservation of tilapia fillets (Ou et al., 2002). Glucose oxidase in alginate coating extended shelf life of winter flounder as compared to coating without enzyme (Field et al., 1986).

2.10.3.1.3.9 Other active packaging systems

Packaging of ready meals in self heating packaging is an important application of active packaging in future. According to EC/450/2009, self heating packaging is packaging with the ability to heat food contents without external heat sources or power. Self heating systems have dual chambers, one surrounding the other. The inner chamber holds the food or drink and the outer chamber contains chemicals that undergo exothermic reaction when combined. Self venting packaging is packaging that controls the steam or pressure in the pack, venting the steam when the required pressure temperature level is reached. Microwavable active packaging is designed to ameliorate the heating behavior of food by shelding, field modification and use of susceptors (Regier, 2014). Microwave susceptors consist of aluminium or stainless steel deposited on substrates like polyester film or on paperboard resulting in even heating, surface browning and crisping (Perry & Lentz, 2009; Ahvenainen, 2003; Kerry et al., 2006). Sira Crisp^{TM} (Sirane Ltd) and SmartPouch® (VacPacInc) are some of the

commercially available microwave susceptors (Sirane, 2011; VacPac, 2014). Steam valves which allow the easy release of steam during microwave cooking are also attached along with the active microwave packs. Flexis^{TM} Steam Valve (Avery Dennison Corp) is a commercial pressure sensitive steam valve that can be applied to most of the flexible food packaging lidding films or moulded containers for the purpose of steaming or cooking convenience food in a microwave oven. It provides a hermetic seal that initially protects the contents of the product and becomes self venting during cooking process. It regulates a gradual temperature balance throughout the cooking process to maintain food quality (Avery, 2011). Incorporation of active packaging technologies into edible coatings is another area that has a potential to grow in future.

2.10.3.2 Intelligent packaging

The aim of food packaging is to protect the food from external contaminants and conventionally concentrated on the safety by restricting the migration of substances from the packaging materials into the food. The concept of internal migration of preservatives to food and the communication function of the package to facilitate decision making are related with intelligent packing (Otlis & Yalcin, 2008). According to EC/450/2009, intelligent materials and articles are those that monitor the condition of packaged food or the environment surrounding the food. Intelligent packaging systems provide the user with information on the conditions of the food or its environment (temperature, pH). It is an extension of the communication function of traditional packaging and communicates to the consumer based on its ability to detect, sense and record the changes in the products environment (Restuccia et al., 2010; Kerry et al., 2014; Realini & Marcos, 2014). In contrary to active components, intelligent components do

not have the intention to release their constituents into the food. The intelligent packaging can also contribute to improving Hazard Analysis and Critical Control Points' (HACCP) and Quality Analysis and Critical Control Points' (QACCP) systems (Heising et al., 2014) which are developed for onsite detection of unsafe food, identify potential health hazards and establish strategies to reduce or to eliminate their occurrence. It also helps to identify processes that strongly affect the quality attributes and efficiently improve the final food quality (Vanderroost et al., 2014). Basically there are three intelligent systems; sensors, indicators and radio frequency identification (RFID) systems (Kerry et al., 2006; Vanderroost et al., 2014)

2.10.3.2.1 Sensors

A sensor can be defined as a device used to detect, locate or quantify energy or matter giving a signal for the detection or measurement of a physical or chemical property to which the device responds (Kress-Rogers, 1998; Kerry et al., 2006). Sensors provide continuous out put of signals. Most of the sensors contain two main functional parts, a receptor and a transducer. Receptors convert the physical or chemical information into energy and transducers measures the energy and convert it into useful analytical signal (Hogan & Kerry, 2008). Sensors are widely used in biomedical and environmental applications (Demas et al., 1999). Sensors provide an alternative to the time consuming and expensive physico-chemical analytical tests to detect the quality and shelf life of food stuffs.

2.10.3.2.1.1 Biosensor

Biosensors are used to detect, record and transmit information pertaining to biological reactions (Yam et al., 2005). Biosensors contain bio-receptors and transducers (Alocilja & Radke, 2003). The bioreceptor

recognizes the target analyte and the transducer converts biochemical signals into quantifiable electronic response (Yam et al., 2005). The bioreceptors may be either organic or biological materials like enzyme, hormone, nucleic acid, antigen, microbes etc. The transducers may be of optical, acoustic or electrochemical. Food Sectinel System® (SIRA Technologies Inc) is a commercial biosensor developed to detect the food pathogens. Specific antibodies are attached to the membrane forming part of the sensor or the barcode. The pathogens cause localized dark bar formation making the barcode unreadable (Yam et al., 2005). ToxinGuard® (Toxin Alert, Canada) is visual diagnostic system based on antibodies printed on polyethylene based plastic packaging material which detect the targeted pathogens such as *Salmonella sp*., *Campylobacter sp*., *E coli*., *Listeria sp*. (Bodenhamer et al., 2004). Pospiskova et al. (2012) developed a biosensor for the detection of biogenic amines formed due to the decarboxylation of amino acids or by amination and transamination of aldehydes and ketones due to microbial action. Biosensors for the detection of xanthine, (adenine nucleotide degradation product in animal tissue) was developed by Arvanitoyannis & Stratakos (2012) by immobilization of xanthine oxide onto the electrodes made of materials such as platinum, silver and pencil graphite (Devi et al., 2013; Dolmaci et al,. 2012; Realini & Marcos, 2014)

2.10.3.2.1.2 Gas sensor

Gas sensors are used for detecting the presence of gaseous analyte in the package. It include oxygen sensors, carbon dioxide sensors, water vapour sensor, ethanol sensor, metal oxide semiconductor field effect transistors, organic conducting polymers and piezoelectric crystal sensors etc. (Kress-Rogers, 1998; Kerry et al., 2006). Papkovsky et al. (2002) have described optical oxygen sensors, such systems are based on the principle of

luminescence quenching or absorbance changes caused by direct contact with the analyte. Optochemical sensors are used to detect the quality of products by sensing gas analyte such as hydrogen sulphide, carbon dioxide and volatile amines (Wolfbeis & List, 1995). The optochemical sensing methods are of three types including fluorescence based system using a pH sensitive indicator, absorption based colourimetric sensing and energy transfer approach using phase fluorimetric detection (Neurater et al., 1999; Mills et al., 1992). pH sensitive dyes can be used to develop sensors for the detection of basic volatile amines in fish, meat and poultry. Indicators based on methyl red/cellulose membrane, curcumin/bacterial cellulose membrane respond through visible colour changes to volatile amines released during fish spoilage (Kuswandi et al., 2012; 2014).

2.10.3.2.1.3 Printed electronics

Printed electronics is an emerging technology on flexible substrates using electrically functional inks. The unique properties of printed electronic sensors include light weight, bendable, rollable, portable and foldable. Possibility of creating sensors on a variety of substrates each shaped and individually tailored to operate uniquely (Vanderroost et al., 2014). The flexible printed chemical sensors contain a receptor printed on top of a printed transducer. Molecular imprinting is another promising technique for selected molecules. The analyte molecules are incorporated into a pre polymeric mixture and allowed to form bonds with the pre polymer. Once the polymer has formed, the analyte molecules are removed leaving the cavity with the analyte molecules shape. The targeted molecule can thus be identified since the shape of the cavity is specific to the molecule of interest (Realini & Marcos, 2014; Kelly et al., 2005).

2.10.3.2.1.4 Chemical sensor

The chemical sensor or the receptor is a chemical selective coating capable of detecting the presence, activity, composition, concentration of particular chemical or gas through surface adsorption. Presence of particular chemicals are being observed and converted into signals by transducer. Transducers are of either active or passive depends on the external power requirement for measurement (Vanderroost et al., 2014).Carbon nanomaterials like nanoparticles, grapheme, graphite, nanofibers and nanotubes are applied in chemical sensors because of their excellent electrical and mechanical properties along with the high specific surface area (Vanderroost et al., 2014). Nano based sensors can be used to detect pathogens, chemical contaminants, spoilage, product tampering, track ingredients or products through the processing chain (Nachay, 2007; De-Azeredo, 2009; Liu et al., 2007). Recent advance in sensors are the use of optical transducers which do not need the electrical power and it can be read out from a distance by using VU, visible or IR light. Silicon based optical transducers are composed of optical circuits which are integrated in silicon semiconductor material (Yebo et al., 2012).

2.10.3.2.1.5 Electronic nose

Electronic nose are other systems used to mimic the mammalian olfactory system within in an instrument designed to obtain repeatable measurements allowing identification and classification of aroma mixtures present in the odour. It generates a unique response to each flavor, odour or savour. Nose system consist of an array of either chemical or biosensors with partial specificity and statistical methods enabling the recognition of flavor, odour or savour (Gardner & Bartlett, 1993; Vanderroost et al., 2014). Electronic nose system was proved to be successful in the quality evaluation

of fresh yellowfin tuna and vacuum packed beef (Blixt & Borch, 1999; Dobrucka & Cierpiszewski, 2014). The aroma emitted by fruits and vegetable can indicate the quality of marketed products. Rajamäki et al. (2004) studied the quality of modified atmosphere packed broiler chicken cuts using electronic nose. The electronic nose results were compared with those obtained by microbiological, sensory and head space gas composition analysis. The e-nose could clearly distinguish the chicken packages with deterioration from fresh packages.

2.10.3.2.2 Indicators

Indicators can be defined as substances that indicate the presence, absence or concentration of another substance or the degree of reaction between two or more substance by means of a characteristic change, especially in colour (Hogan & Kerry, 2008). In contrast with sensors, indicators do not provide any information about the quantity and data of measurement (Vanderroost et al., 2014). They provide visual and qualitative information of the packaged food by means of colour changes (Kerry et al. 2006). The basic requirement of an indicator is that the colour and intensity of indicator should be irreversible; otherwise, this may cause possible false information (Pavelkova, 2012)

2.10.3.2.2.1 Freshness indicator

Freshness indicators provide the product quality information resulting from microbial growth or chemical changes within a food product. The reaction between the microbial growth metabolites and the integrated indicators within the package provide visual information regarding the microbial quality of the product (Kerry et al., 2006; Kuswandi et al., 2013). In 1999, COX Technologies, USA launched FreshTag® colourimetric

indicator labels that react to volatile amines produced during storage of fish and seafood products (Hogan & Kerry, 2008), however the product was discontinued in 2004 (Kerry, 2014; Realini & Marcos, 2014). A colourimetric chitosan based pH indicator was developed by Yoshida et al. (2014) with a potential to be used as indicators of metabolites derived from microbial growth such as n-butyrate, L-lactic acid, D-lactate and acetic acid. Carbon dioxide produced in meat products during storage is also an indication of food spoilage. Carbon dioxide indicators were developed by the researchers of Sejong University consisting of aqueous solutions of chitosan or whey protein isolate. The carbon dioxide presence was detected by the changes in transparency by the pH dependent whey (Jung et al., 2012; Lee & Ko, 2014). The disadvantage of freshness indicator on colour changes are colour changes indicating contamination can occur in products free from any significant sensory or quality deterioration. The presence of certain target metabolite is not necessarily an indication of poor quality (Hogan & Kerry, 2008)

2.10.3.2.2.2 Time temperature indicator (TTI)

Temperature is one of the most important environmental factors determining the kinetics of physical, chemical and microbial spoilage in food products. According to EC/450/2009, Time temperature indicators are meant to give information on whether a threshold temperature has been exceeded over time and or to estimate the minimum amount of time a product has spent above the threshold temperature (time temperature history). Basically TTIs are small tags or labels that keep track of time-temperature of a perishable commodity from the point of production to the end consumer (Fu & Labuza, 1995). These labels provide visual indications of temperature history during the distribution, and storage. Therefor they can inform about the temperature

abuse for chilled or frozen products. Three basic types of TTI are available in market: critical temperature indicators, partial history indicators and full history indicators (Singh, 2000; Yam et al., 2005). Currently available commercial TTIs are based on diffusion, enzymatic and polymer systems. 3M Monitor Mark® and Freshness Check® of 3M Company, USA are commercial diffusion based time temperature indicators. The VITSAB® (Vitsab USA and Vitsab International, Sweden) is an example of commercial enzymatic TTI in which a colour change induced by a drop in pH resulting from the controlled enzymatic hydrolysis of a lipid substrate. Lifelines Freshness Monitor®, Fresh-Check (lifelines Technology Inc, USA) are temperature dependent polymerization reaction TTIs. OnVu^{TM} (Ciba Speciality Chemicals, Inc, Switzerland) contain benzopyridines, an organic pigment that change colour with time at rates determined by temperature (Hogan & Kerry, 2008). The indicator is activated by exposure to UV light to become dark blue and the colour gradually fades with time (O'Grady & Kerry, 2008). FreshCode (Varcode Ltd) and Tempix (Tempix AB, Sweden) are based on barcodes printed with fading inks that disappear due to temperature abuse. Tempix indicator works in the range of -30^0C to $+30^0$C temperature range and is accurate to 0.5^0C intervals (Tempix, 2014; Varcode, 2014).

2.10.3.2.2.3 Integrity indicator

Leak indicators to package ensure package integrity throughout the production and distribution chain. Visual oxygen indicators in MAP foods with low initial oxygen are studied by Davies & Gardner, 1996; Mattila-Sandholm et al., 1995). Visual oxygen indicators with redox dyes change its colour with changes in oxygen concentration. Disadvantage of such system is that the device should be highly sensitive and the residual

oxygen in the package are susceptible to indicators. Oxygen comes through leakage may also be consumed by the natural microbes present in the food (Mattila-Sandholm et al., 1998). The colour change is reversible and returns to the original form if the oxygen concentration is reduced (Hurme, 2003; Realini & Marcos, 2014).

Ageless Eye® (Mitsubishi Gas Chemical company) is oxygen indicator tablets which indicate the presence or absence of oxygen by colour change. It indicate the lack of oxygen (<0.01%) by turning pink. At an oxygen level of 0.5% or more, the tablet turns blue. The presence of oxygen will be indicated in five minutes or less, while the change from blue to pink may take three hours or more (Mitsubishi Gas Chemical, 2016). EMCO Packaging, (UK) has launched reversible and non reversible oxygen indicator labels for the visual indication of pack integrity (Emco packaging, 2013).

2.10.3.2.2.4 Radiofrequency identification (RFID)

Radiofrequency identification (RFID) is an automatic identification technology that uses wireless sensors to identify items and gather data without human intervention. An RFID is based on tags and readers (Tajima, 2007; Hong et al., 2011). Most RFID tags store some sort of identification number based on which reader can retrieve information about the ID number from a database and acts upon it accordingly (Todorovic et al., 2014). RFID tags are of two categories; passive and active. Passive tags rely on the power supplied by the reader. When radio waves from the reader are encountered by a passive RFID tag, the coiled antenna within the tag forms a magnetic field. The tag draws energy from it and sends the information encoded in the tags memory. Semi passive RFID tags use battery to maintain memory in the tag or power the electronics that enable the tag to modulate

the Electromagnetic waves emitted by the reader antenna. Active RFID tags are powered by an internal battery, used to run the microchips circuitry and to broadcast a signal to the reader (Vanderroost et al., 2014)

RFID has been successfully applied to traceability control and supply chain management processes because of its ability to identify, categorize and manage the flow of goods (Jones et al., 2004; Sarac et al., 2010; Ruiz-Garcia & Lunadei, 2011). Studies indicate that RFID is more advanced than the zebra black and white paper; barcode system for food traceability (Jedermann et al., 2009). It provides supply chain visibility, which enables fast automated processes at the supply chain such as exception management and information sharing (Tajima, 2007). Mountable, non integrated and no flexible sensor based RFID with tags are available in the market to monitor the temperature, relative humidity, light exposure, pressure and pH of products. These tags detect the possible interruptions of cold chain which are harmful to the food quality and safety (Vanderroost et al., 2014).

2.10.3.3 Legal aspects of intelligent packing.

Article 3 of EC/1935/2004 states that food contact materials should not transfer constituents to food in quantities that could endanger human health. Substances that bring about an unacceptable change in the composition and substances that bring about deterioration in organoleptic characteristics. Commission regulation No 450/2009 states that the individual substances or group/combination of substances which make up the active or intelligent component should be safe and comply with the requirements of the framework regulation No. EC/1935/2004 and regulation no EC/450/2009. Articles 4(d) and 11 of EC No 450/2009 specify that active and intelligent materials should be labeled as non-edible to avoid the accidental

consumption. The labeling also should not mislead the consumer. Articles 12 and 13 specify that information should be provided throughout the package chain including consumer to ensure the correct use of these materials and articles.

Table 2.6. Commercially available intelligent packing systems (Biji et al.,2015)

Trade Name	Manufacturer	Type
O_2 Sense ™	Freshpoint Lab	Integrity Indicator
Novas®	Insignia Technologies Ltd	Integrity Indicator
Ageless Eye®	Mitsubishi Gas Chemical Inc	Integrity Indicator
Freshtag®	COX Technologies	Freshness Indicator
Sensorq®	DSM NV And Food Quality Sensor International	Freshness Indicator
Timestrip Complete®	Timestrip UK Ltd	Time Temperature
Timestrip®PLUS Duo	Timestrip UK Ltd	Temperature Indicator
Monitormark™	3M™, Minnesota	Time Temperature Indicator
Fresh-Check®	Temptime Corp	Time Temperature Indicator
Onvu™	Ciba Specialty Chemicals And Freshpoint	Time Temperature Indicator
Checkpoint®	Vitsab	Time Temperature Indicator
Cook-Chex	Pymah Corp	Time Temperature Indicator
Colour-Therm	Colour Therm	Time Temperature Indicator
Thermax	Thermographic Measurements Ltd	Time Temperature Indicator
Timestrip®	Timestrip Ltd	Integrity Indicators
Novas®	Insignia Technologies Ltd	Integrity Indicators
Easy2log®	CAEN RFID Srl	RFID
Intelligent Box	Mondi Plc	RFID
CS8304	Convergence Systems Ltd	RFID
Temptrip	Temptrip LLC	RFID

2.10.3.4 Future trends

Many of the active packaging technologies increase shelf life of fish only marginally and usually not the initial prime quality. A better effect can be obtained by maintaining the raw material quality, i.e. reducing the initial spoilage counts or lowering the storage temperature by one or two degrees. Combining active packaging with super chilling (sub-zero (-1 to -2ºC) storage). Active packaging has therefore a greater potential to be a

success for fish products with added value, for example, fish based ready meals. This is also possibly the segment with highest growth potential. Adding value to the raw material will drive increased seafood consumption. These innovations will have to be supported by packaging that incorporates convenience just as is offered by many minimally processed prepared foods. Quality and production management of fish as raw material on storage is of great importance since fish is a highly perishable commodity. Supplies of fish are unstable and fresh fish can be stored only for a short time. Freshness is one of the most important aspects of fish and fishery products (Olafsdottir et al., 1997)

2.10.4 Packaging materials used for active packaging

Selection of packaging material is essential to maintain the quality and safety of active packed foods. Flexible and semi rigid containers, plastic laminates are used for active packaging of food materials. Light weight, heat sealability, strength etc are some of the properties which make the packaging material suitable for active packing. Plastic materials may be of monolayer or multilayer formed from several layers of different plastics. By lamination or co extrusion, it is possible to design a plastic material with all desired properties required for a package. The most popular flexible plastic laminates are made from polyethylene (PE), polypropylene (PP), polyamide (nylons), polyethylene terephthalate (PET), polyvinyl chloride (PVC) ethylene vinyl alcohol (EVOH). Rigid and semi-rigid structures are commonly produced from PP, PET, unplasticized PVC and expanded polystyrene.

2.10.4.1 Ethylene vinyl alcohol

EVOH is copolymerized with ethylene to produce EVOH and it is widely used as a gas barrier layer in active and modified atmospheric packaging. It has good processing properties and therefore suited for conversion into plastic films and structures. EVOH process high mechanical strength, high resistance to oils and organic solvents and it has high thermal stability (Gopal, 2007).

2.10.4.2 Polyethylenes

Polyethylene is the simplest group of synthetic polymers and it is the most commonly used plastic films, composed of a carbon backbone with a degree of side chain branching which influences density. Low density polyethylene (LDPE) (density, 0.910–0.925gcm^{-3}) is generally used in film form, whereas high density polyethylene (HDPE) (density, 0.940 g cm^{-3}) is commonly used for rigid and semi rigid structures. The hydrophobic nature of PE makes them good barrier towards the water vapour but PE has low gas barrier properties. PE melts at a relatively low temperature ranging from approximately 100-120^{0}C. A less branched variant called linear low density polyethylene (LLDPE) which offers good heat sealing properties is used as a sealant layer to impart heat sealing properties on base trays and lidding films (Gopal, 2007).

2.10.4.3 Polyamides

Polyamide or nylon has high tensile strength, good puncture and abrasion resistance and good gas barrier properties. Nylons are generally moisture sensitive (hydrophilic) and will absorb water from their environment. They are relatively strong and tough which make them suitable for application in vacuum packaging (Gopal, 2007).

2.10.4.4 Polyethylene terephthalate

Polyethylene terephthalate is the most common polyester used in food packaging applications. PET is a good gas and water vapour barrier. PET is strong, offers good clarity and is temperature resistant. Crystalline PET (CPET) has poorer optical properties but improved heat resistance melting at temperatures in excess of 270^0C. High temperature resistance make it ideal for microwave applications (Gopal, 2007).

2.10.4.5 Polypropylene

Polypropylene is used in flexible, rigid and semirigid packaging structures. Rigid trays made of PP are used for active packaging applications. The melting point of PP is approximately at 170^0C. It is used for microwaving low fatty food material. It should not be used for microwaving fatty foods, where temperatures in excess of its melting point could be reached (Gopal, 2007).

2.10.4.6 Polystyrene (PS)

Expanded PS (EPS) is used as a base tray for over wrapped fresh meat, fish and poultry products. The high gas permeability of foamed PS requires the material to be laminated to a plastic such as EVOH that provides the required gas barrier properties (Gopal, 2007).

2.10.4.7 Polyvinyl chloride

Polyvinyl chloride has a relatively low softening temperature and good processing properties and is therefore an ideal material for producing thermoformed packaging structures. Although a poor gas barrier in its plasticised form, unplasticised PVC has improved gas and water vapour barrier properties which can at best be described as moderate. Oil and grease resistance are excellent, but PVC can be softened by certain organic solvents.

It is a common structural material in MAP thermoformed base trays, where it is laminated to PE to provide the required heat sealing properties (Gopal, 2007)

2.10.4.8 Polyvinylidene chloride (PVdC)

Polyvinylidene chloride (copolymer of vinyl chloride and vinylidene chloride) possesses excellent gas, water vapour and odour barrier properties, with good resistance to oil, grease and organic solvents. Unlike EVOH, the gas barrier properties of PVdC are not significantly affected by the presence of moisture. PVdC effectively heat seals to itself and to other materials. The high temperature resistance enables uses in packs for hot filling and sterilization processes (Gopal, 2007).

2.11 Concluding remarks

Various innovative packaging technologies are developing in recent years, which are being integrated to the packaging systems to meet the requirements of food supply chain. Adoption of suitable packaging technologies by the food industry can be useful to extend the shelf life, improve quality, safety, and provide information about the product. Research on these emerging packaging technologies can result in further improvement of the existing system. In future untapped opportunities exist for smart packaging to offer consumer benefits and convenience.

Chapter -3
MATERIALS AND METHODS

Contents

3.1 Materials

3.1.1 Fish

The commercially important yellowfin tuna of average weight of 7.5± 1.4 kg collected from local fish landing centre, Cochin, India was used for the study. The average body length of the fish was 71±3.8cm with a width of 20±1.4cm. The samples were brought to the laboratory in chilled condition in order to maintain the quality of the sample. Samples were kept on ice (0-1 ^{0}C) until used.

Plate 3.1 Yellowfin tuna (*Thunnus albacares)* used for the study

Plate 3.2 Yellowfin tuna meat

Plate 3.3 Commercial oxygen scavenger used for the study

3.1.2 Chemical and preservatives

The chemicals and preservatives used for the study were of AR grade, from the manufactures of Sigma Aldrich (St. Louis, Missouri), Merck (Merck & Co. Inc. NJ, USA), Fisher Scientific (USA), Qualigens (Qualigens Fine Chemicals, Mumbai, India). Only good grade and generally regarded as safe (GRAS) preservatives were used in the study.

3.1.3 Bacteriological Media

Dehydrated bacteriological media and supplements from the manufactures of Difco (Voigt Global Distributio, Inc., Lawsence, KS, USA) Hi Media (HiMedia Laboraatories, Pvt Ltd., Mumbai, India) and Oxoid (Cambridge, UK) were used in the study

3.1.4 O_2 Scavenger

Commercial O_2 scavenger sachets (Ageless® ZPT 200 EC O_2 absorber) with absorption capacity of 200 ml supplied by Sealed air (India) Pvt. Ltd, Bangalore, India, were used in the study. The sachet contains reactive compounds which are packed in a small pouches of paper coated with perforated polypropylene. The pouches are highly permeable to gas and water vapour, and are resistant to damage and labeled "do not eat". The scavenger is self activated type and the scavenging action is based on the principle of iron powder oxidation

3.1.5 Packaging materials

Multilayered Ethylene Vinyl Alcohol (EVOH) film (nylon, EVOH and polypropylene) supplied by Sealed Air (India) Pvt. Ltd, Bangalore, India, of thickness 0.14mm were used for the study. High Molecular weight high density polyethylene (HM-HDPE) film was used to prepare dual action sachets.

3.2 Methods

3.2.1 Physico-chemical properties of packaging materials

The packaging materials used for the study were tested for various physico chemical properties for its suitability. Before testing, all the samples were conditioned at 64% relative humidity at 25 ± 2^0C for 24 hours using REMI programmable environmental test chamber, Model No. 412 LAG supplied by Rajendra Electriacal Industries, Vasi, India.

3.2.1.1 Gas transmission rate (GTR)

O_2 and CO_2 permeability of the film was carried out by using gas permeability apparatus (Gas and stream permeability, Ats Faar, Societa' Per Azioni, Milano, Italia) (ASTM, 1975). The test material was cut into suitable size (10cm diameter). B, C and D valve of the instrument was opened and the upper half of the permeability cell was removed. A dried circular filter paper (Whatman No. 1) was placed on the top of the insert after applying vacuum adhesive grease and the sample of film was spread over the filter paper. An added mass was placed into the mould. The upper part of the permeability cell was then replaced. All the valves (A, B, C) were closed and the vacuum pump was turned on. Then valve C was opened to create vacuum in the lower portion and it was checked by tilting central vacuum gauge (0.2mm Hg). The D valve was then opened for purging and A valve for removing the atmospheric gas if any. Then both A and D valves were closed. Mercury (Hg) was transferred into the cell by tilting the outer portion and allowed for few minutes to attain 0.2 mm Hg vacuum. Valve A was opened to apply the test gas (CO_2 or O_2) and the pressure was adjusted using the gas cylinder valve. Timer was tuned on and allowed 15 min for stabilization. The initial vacuum reading was noted from the vacuum gauge or in the Eurotherm Chassell and

final reading was noted at a particular interval and the gas transmission rate was calculated and expressed as ml $m^{-2}24h^{-1}$at 1 atm pressure.

3.2.1.2 Water vapour transmission rate (WVTR)

Water vapour transmission rate was analyzed by the method of ASTM (1987). The packaging material was cut using a template to fit into the inner annular recess of the aluminum dish (50 cm^2). The dish was filled with desiccant (fused calcium chloride) up to 1 to 2 mm of the supporting ring and the test piece was then placed on the supporting ring and was centered. The waxing template was placed centrally over the dish and test piece and molten wax was poured into the annular recess until the wax was leveled with the top surface of the template. Air bubbles in the wax were broken with a small gas jet and the wax was allowed to harden and the template was removed. Filling and sealing of the dish was carried out as rapidly as possible to minimize the water absorption of desiccant from atmosphere. Care was taken not to damage the test area during the operation. A thin layer of petroleum jelly was applied to the template edges to facilitate the easy removal of the template from the wax. The open end of the dish containing the desiccant and test specimen was selected and the dish was exposed to the desired relative humidity (RH) and temperature conditions (37^0C and 92% RH, when the desiccant used exerts 2% RH). The required members of dishes prepared and were placed in the humidity cabinet. Weighing at suitable intervals was carried out which must be sufficiently frequent to complete the test before the relative humidity of the dish rises above 2%. Increase in weight of the desiccant over a known period of time gives the amount of water vapour transmitted by the specimen and expressed as $gm^{-2}24h^{-1}$ at 90± 2 RH at 37^0C.

3.2.1.3 Overall migration study

Overall migration test was carried as per Indian standard (IS: 9845, 1998). For the determination of migration of constituents from plastic materials, which comes in contact with food stuffs, the choice of stimulating solvents and test conditions (time temperature) depends on the type of food and condition of use of food products. The simulating solvents used for the study were n-heptane (38^0C for 30 min) and distilled water (40^0C for 10 days)

The pouches used for the study were rinsed with water (25-30^0C) to remove extraneous materials prior to actual migration test. The pouches were filled to their maximum filling capacity with preheated stimulant at test temperature and were sealed after excluding air present in the pouch as much as possible. The filled pouches were exposed to specified temperature maintained in the incubator/water bath for the specified duration of time. After that, the pouches were removed and the contents were transferred immediately into a clean pyrex beaker along with three washings of the specimen with small quantity of fresh stimulant. The contents in pyrex beaker was evaporated to about 50-60 ml and transferred to a clean stainless steel dish along with three washings of pyrex beaker with small quantity of the fresh stimulant. It was then evaporated in an oven at $110^0 \pm 5^0$C to concentrate the constituents in the dish. The dish with extractives was cooled in desiccators for 30 min and weighed to nearest. 0.1 mg till the content weight of residue was obtained. The extractives was calculated as below and expressed as $mg.l^{-1}$ or $mg.dm^{-2}$ In case of heptanes as solvent, the amount of extractives obtained was divided by a factor of five in arriving at the extractives for a food product.

Amount of extractives = (M/V) X 1000 (ppm),

Where,

M = mass of residue in mg - blank value

V = total volume in ml of stimulant used in each replicate

Overall migration residue in $mg.dm^{-2}$ =

(mass of residue in mgx100$mg.dm^{-2}$)/area exposed in cm^2

3.2.1.4 Tensile strength and elongation at break

The tensile strength and elongation at break was determined by using Universal Testing Machine (Lloyd instruments LRX plus, UK) (IS: 2508-1984). Ten packaging material samples were cut in to strips of suitable size (15 mm width X 50 mm length) in both machine and cross direction and one end of the stripe were tightly gripped in the upper clamp and the other end in the lower clamp after adjusting the alignment as straight as possible. The machine was switched on at the pre-adjusted speed (500 mm/min). The load range used was such that the breaking load of the test pieces falls between 15-85% of full-scale reading. The result of each individual strip was recorded and the tensile strength at break was calculated and expressed as kg cm^{-2} from the original areas of cross section. The elongation at break was calculated and was expressed as % of the original length.

3.2.1.5 Heat seal strength

The packaging materials heat sealing strength was determined by measuring the force required to pull apart the pieces of sealed film (ASTM, 1973) using universal testing machine (Lloyd instruments LRX plus, UK). The samples were cut into suitable size (15 mm width X 50 mm length) and clamped between the jaws. The machine was switched on at the pre adjusted speed (200 mm/min). The maximum stress applied to the sample at breakage

was noted as heat seal strength. Heat seal strength of both machine and cross direction was recorded from the minimum of 10 readings and the results were expressed as kg cm^{-2}

3.2.2 Biochemical analysis

3.2.2.1 Proximate composition

a. Moisture

A known weight of homogenized sample (10g) was taken in a pre weighed clean petri dish. The sample was evenly spread on the dish and placed in a hot air oven at 105 ± 1^0C for 16 hours. Samples were cooled in desiccators and weighed until a constant weight was obtained and moisture content was calculated and expressed as percentage (AOAC, 2000).

b. Ash

About 1-2 g of moisture free sample was transferred to a preheated cooled and weighed silica dish and the sample was carbonized by burning at low red heat and was placed in a muffle furnace at 550^0C for about 4 hours until a gray/white ash was obtained (AOAC, 2005). It was then cooled in desiccators and weighed and percentage of ash was calculated.

c. Crude fat

Crude fat content was analyzed by taking about 2-3 g of accurately weighed moisture free sample in a thimble plugged with cotton. The extraction thimble was placed in the extraction unit of Soxhlet apparatus with an attached previously weighed receiving flask. Petroleum ether of boiling point 40-60^0C was poured washing into thimble through a glass funnel. The extraction unit and receiving unit was then connected to the

Soxhlet condenser. The flask was heated on a boiling water bath. The extraction was continued at a condensation rate of 5-6 drops per second till the solvent in the extraction unit becomes clear (10h). After completing the extraction the flask was removed, dried in an oven at 100 ± 1^0C and weighed. The crude fat was calculated and expressed as percentage (AOAC, 2005).

d. Crude protein

Crude protein content was analyzed by digesting and distilling the sample (AOAC, 2000). About 0.5-1g of the moisture free fish sample was transferred into a Kjeldahl flask of 100 ml capacity. A few glass beads and a pinch of digestion mixture [K_2SO_4:CuSO4 (8:1)] and 20 ml concentrated sulphuric acid were added. It was digested over a heating coil until the solution turned colourless.

To the digested and cooled solution, distilled water was added in small quantities with intermittent shaking and cooling until the addition of water did not generate heat. It was transferred quantitatively into a 100 ml standard flask and the volume made up to 100ml. About 2-5 ml of the made-up solution was transferred to the reaction chamber of the Micro-Kjeldahl distillation apparatus. Five ml of 40% sodium hydroxide solution and two drops of phenolphthalein indicator were added and washed with distilled water. Distillation was carried out and the ammonia liberated was collected in a 100 ml conical flask containing 10 ml 2% boric acid with few drops of Tashiro's indicator. Distillation was continued for 4 min once the solution turns from pink to green colour and the amount of ammonia liberated was determined by titrating with 0.01 N standard sulphuric acid. Crude protein content was calculated by multiplying total nitrogen content with conversion factor of 6.25 and expressed as percentage.

3.2.2.2 Volatile base compounds

Preparation of Trichloro-acetic acid (TCA) extract

About 10 g of accurately weighed homogenized sample was extracted thrice with 10% Trichloro acetic acid (TCA) by grinding in a mortar and pestle. 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. This extract was used to measure total volatile base nitrogen and trimethyl amine nitrogen.

a. Total Volatile Base Nitrogen

Total volatile bases in the samples were determined as TVB-N by micro diffusion method (Conway, 1962). 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 and the glass lid applied with vacuum grease was covered over it, leaving a small space. Through this space, 1 ml of saturated potassium carbonate was added and the unit was sealed immediately with the glass lid. It was then rotated slowly to mix the contents and kept undisturbed overnight. The amount of unreacted acid in the inner chamber was determined by titrating against N/100 sodium hydroxide using Tashiro's indicator (methylene blue 0.1% and methyl red 0.03% in ethanol). Similarly a blank was also run using 10% TCA instead of sample extract. TVB-N was calculated and expressed as mg $N_2 100g^{-1}$ of the sample.

b. Tri-methyl amine-nitrogen

TMA was determined as tri methyl amine nitrogen by the micro diffusion method (Conway, 1962). 1 ml of standard N/100 sulphuric acid was added in the inner chamber of diffusion unit. To the outer chamber 1ml of

TCA extract was added followed by 1ml neutralized formaldehyde. This was kept 3 min to ensure the binding of formaldehyde with all the primary and secondary amines and ammonia contained in the extract. To this, 1 ml saturated potassium carbonate was added and the analysis was further carried out as explained in TVB-N determination. TMA-N was calculated and expressed as mg $N_2 100g^{-1}$ of the sample.

3.2.2.3 Lipid oxidation – hydrolysis products

a. Thiobarbituric acid (TBA) value

About 10 g of homogenized fish sample was mixed with 100 ml 0.2N HCl and homogenized to slurry. This slurry was then poured in to a round bottom flask and connected to the TBA distillation apparatus. Distillation was done to collect 50 ml of the distillate within 10 minutes. 5 ml of the distillate was taken in the test tube and 5 ml of TBA reagent (0.288 g TBA reagent in 100 ml acetic acid) was added and heated in boiling water bath for 30min. A blank was carried out with distilled water. Colour developed was measured in a spectrophotometer at 538nm and TBA value was determined and expressed as mg malonaldehyde kg^{-1} of fish sample (Tarladgis et al., 1960).

b. Peroxide value

Preparation of chloroform extract

About 10 g of the homogenized fish sample was taken into a mortar and mixed with anhydrous sodium sulphate to remove the moisture content. It was transferred to an iodine flask and to this 100 ml of chloroform was added, mixed well and kept under dark for overnight.

Chloroform extract was filtered into another iodine flask and 20 ml was taken in a small beaker to find out the amount of fat present in it. For analyzing the peroxide value, 20 ml of chloroform extract was taken in an iodine flask. To this 30 ml of glacial acetic acid and a pinch of potassium iodide were added. Stopper was inserted immediately and shaken for 1 min and left exactly for 30 min in dark at ambient temperature. 50 ml of distilled water was added and liberated iodine was titrated with 0.01 N sodium thiosulphate solution using starch as indicator until blue colour disappears. Test was done in triplicates and blank was also carried out using chloroform. PV was calculated and expressed as milliequlvalant of O_2 Kg $^{-1}$ fat (AOCS, 1989).

c. Free fatty acid

For this, 20 ml of chloroform extract was taken in a conical flask and placed on the water bath to evaporate chloroform completely. To this 10 ml of neutralized alcohol (few drops of phenolphthalein indicator was added to the absolute alcohol and to that 1 N NaOH was added till it turns slightly pinkish) and kept on water bath for few minutes to dissolve the fat. It was cooled and titrated with 0.01 N NaOH using phenolphthalein indicator till it turns slightly pinkish and FFA was calculated and expressed as mg% oleic acid (AOCS, 1989)

3.2.2.4 ATP breakdown products

ATP breakdown products were determined according to the method of Ryder (1985) using High Performance Liquid Chromatography (HPLC). A shimadzu prominence HPLC system consisting of LC20AD quaternary gradient pump ,SPD 20A UV-VIS detector, Rheodyne 7725i manual injector and CBM-20A controller and C18 stainless steel colum was

used for the study. Data acquisition and analysis was perfprmed by LC solution software.

a. Standard preparation

The standard nucleotide solution was prepared individually so as to give a concentration of 10mM. For this 137.79 mg ATP, 106.8mg ADP,86.8mg AMP, 134.07mg IMP,67.02mg inosine, and 34.03mg Hx in 0.1N NaOH were dissolved in HPLC grade water and made up to 25 ml using milli Q purified distilled water. From this 10mM stock solution, 0.01, 0.05, 0.1, 0.5 and 1.0mM mixed standard solutions were prepared by diluting with milli Q purified distilled water and used for obtaining standards curve area.

b. Sample preparation

Fish extract was prepared by homogenizing 5g of fish muscle (without skin) with 25 ml chilled 6M perchloric acid in a tissue homogenizer (Mic Cra D 8Si 13024, ART Modern Labortechnik, Germany) at 0^0C for 1 min. The homogenate was centrifuged at 6500 rpm for 20 min at 4^0C. The supernatant was then decanted and immediately neutralized to pH 6.5-6.8 by using 1M potassium hydroxide solution. After standing for 30 min at 1-2^0C, the precipitated potassium perchlorate was removed by filtration through a syringe of pore size 0.45 µm. The filtrate was stored at -20^0C until analyzed.

High Performance Liquid Chromatography (Shimadzu prominence HPLC system) was used for quantitative analysis of ATP breakdown compounds of prepared samples. Twenty microliter aliquots of the sample extracts were injected into the HPLC and separation of the nucleotide products were achieved by a 5µm pore size column (C18 stainless steel column with size 250 X 4.6mm length x dia). The mobile phase of 0.04

M potassium dihydrogen orthophosphate and 0.06M dipotassiun hydrogen orthophosphate dissolved in milli Q purified distilled water was used at a flow rate of 1.5 $mlmin^{-1}$. The peaks obtained from fish muscle products, comprising ADP, AMP, IMP, Hx, HxR were measured and K, Ki, H, G, P and Fr values were calculated using the formula described by Burns et al. (1985), Gill et al. (1987), Karube et al. (1984), Shahidi et al. (1994), Luong et al. (1992) and Saito et al. (1959) respectively.

K value (%) = [(HxR+Hx)/(ATP+ ADP+AMP+IMP+Hx+HxR)]X100.

Ki value (%) = [(Hx+HxR)/(IMP+ Hx+HxR)]X100

H value (%) = [(Hx)/(IMP+Hx+HxR)]X100

G value (%) = [(Hx+HxR)/(AMP+IMP+HxR)]X100

P value (%) = [(HxR+Hx)/(AMP+IMP+HxR+Hx)]X 100

Fr value (%) = [(IMP)/(IMP+HxR+Hx)]X100

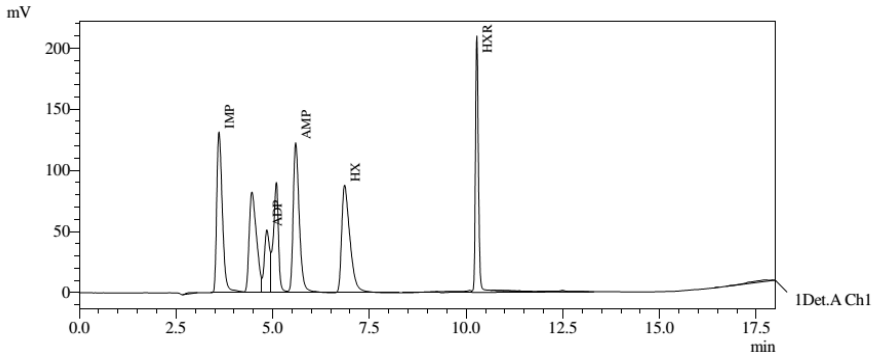


Fig. 3.1. HPLC chromatogram for a standard mixture of nucleotide breakdown products

3.2.2.5 Biogenic amines

The biogenic amine content in fish was determined using rapid HPLC method as described by Özogul et al. (2002b). The gradient system and the flow rate were modified depending on the retention time of the standard amine solution to get good resolution with a short time.

a. Preparation of standard amine solutions

Putrescine dihydrochloride 182.9mg, cadaverine dihydrochloride 171.4mg, spermidine trihydrochloride 175.3mg, spermine tetrahydrochloride 172.0 mg, histamine dihydrochloride 165.7mg and tyramine hydrochloride 126.7 mg were dissolved separately in 10 ml HPLC grade water. It was again diluted ten times to get the final concentration of the free base for each amine to 1mg ml^{-1} solution. From this 2.5, 6.25, 12.5 and 18.75µl of each standard was taken and made up to 250µl using HPLC grade water and derivatized to get a concentration of 10, 25, 50, and 75 $\mu g ml^{-1}$ (ppm) respectively. From 100ppm stock solution, 320 µl, 160 µl, 80 µl and 40 µl were made up to 1000 µl using HPLC grade water and derivatized to get 32, 16, 8 and 4 ppm respectively.

b. Preparation of sample

Fish muscle (5g) was taken from the dorsal part of the fish without skin and transferred to a centrifuge tube. The sample was homogenized with 25 ml 6% TCA for 3min, centrifuged at 12000 rpm for 10 min at 4^0C and filtered through Whatman No. 1 filter paper. The aliquot was made up to 25 ml with 6% TCA and stored at -20^0C until analysis.

c. Derivatization procedure

A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance the reaction with amines. For Derivatization of standard amine solutions, 50µl was taken (2 ml for extracted fish samples) from each free base standard solution (10 mg/ml). One milliliter of 2 M sodium hydroxide was added, followed by 1 ml benzoyl chloride (2%) and mixed on a vortex mixer for 1 min. The reaction mixture was left at room temperature (24^0C) for 30min. The benzoylation was stopped by adding 2 ml

of saturated NaCl solution. The solution was then extracted two times with 2 ml of diethyl ether (1ml each from two steps). The upper organic layer was transferred into a clean tube after mixing and evaporated to dryness in a stream of nitrogen using vacuum drier. The residue was dissolved in 500 µl of acetonitril and 5µl aliquots was injected into HPLC

d. Chromatographic conditions

Chromatographic separation made use of continuous gradient elusion with acetonitril (eluant A and HPLC grade water (eluant B). The gradient started at 50% acetonitril and was increased to 80% in 8 min. The flow rate was 1.5ml/min and injection volume was 20µl. The total separation time was less than 7 min and the gradient was run for 15 min to ensure full separation. Detection was monitored at 254 nm. Calibration curve for each amines were prepared and correlation coefficient of peak areas against amines standard concentration for each compound was calculated.

Table 3.1 Chromatographic conditions used for biogenic amine study

Time (min)	Acetonitrile (%)	Water (%)	Flow rate (ml min^{-1})
0	50	50	1.5
8	80	20	1.5
15	50	50	1.5

The quality index and biogenic amine index were calculated according to the procedures described by Mieltz and Karmas (1977), Veciana-Nogues et al (1997) respectively. The formuas used were as follows.

Quality index = (Histamine + putrescine + cadaverine)/(1 + spermidine + spermine)

Biogenic amine index (BAI)= (histamine+putrescine+cadaverine+ tyramine).

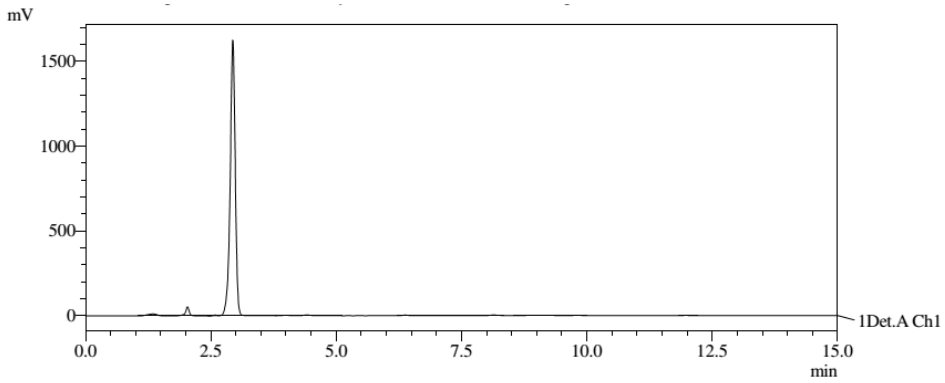


Fig. 3.2. HPLC Chromatogram of benzoylated putrescine standard

3.2.3 Physico - chemical analysis

3.2.3.1 Analysis of the head space gas composition

The gas composition inside the packages were analyzed by using a gas analyzer (PBI Dansensor, Checkmate 9900, Ronnedevej, Denmark) which functions based on a solid state O_2 ion conductive material (Zirconium oxide). Gas analysis was performed by drawing head space gas sample by piercing the syringe needle through a rubber septum glued on the film.

3.2.3.2 pH

The pH was measured by homogenizing the fish sample in distilled water (1:2 w/v) by using a glass electrode digital pH meter (Cyberscan 510, Eutech Instruments, Singapore) as described in IS: 2168, (1971).

3.2.3.3 Drip loss

Drip loss of the samples was measured gravimetrically by taking the weight difference of the sample with and without exudate. The exudate was removed by draining and wiping the sample with filter paper (Mohan, 2008).

3.2.3.4 Water holding capacity

Water holding capacity (WHC) was measured by placing sample of 1cm^3 on a pre weighed filter paper and applying a pressure of 1 KN for 60s using a compression tester (Gaydon compressor tester, Model No 936, H.E. Messer Ltd., London UK). The difference in weight was expressed as WHC.

3.2.3.5 Texture profile analysis (TPA)

The texture profile analysis was measured using the method described by Bourne (1978) by using a universal testing machine (Lloyd instruments, LRX Plus, Lloyd Instruments Ltd, Hampshire, UK). The load cell used was a cylindrical probe of 50mm diameter equipped with a sensor of 50N. Samples were cut into uniform size of 2cm^3 from each pack and were used for the analysis. The texture measurement was composed of two consecutive compressions (40%) of the sample at a crosshead speed of 12 mm/min. The basic principle behind the texture analyzer is the probe forced into the specimen, a shearing force acts which causes the sample to deform or rupture. This data from each test was used to calculate mean value for the TPA parameters. The values for hardness 1 and 2 (resistance at maximum compression during the first and second compression), cohesiveness, the extent to which the sample could be deformed before rupture (ratio of the positive force area during the 2nd compression to that during the 1st compression. Springiness, the ability of the sample to recover its original form after the deforming force is removed (the ratio of the time duration of force input during the 2nd compression to that during the 1st compression) and chewiness, the work needed to chew a solid sample to a steady state of swallowing (hardness 1 X cohesiveness X springiness in kg) where determined

3.2.3.6 Colour

Colour of the fish sample was determined with Hunter's colourimeter (Hunter Lab colourimeter, MiniScan® XE Plus Hunter Associates Lab inc., Reston, Virginia, USA), calibrated with a white tile having standard values of X = 81.0, y = 85.8 and z= 91.2 with corresponding L*, a* and b* values of 67.81, 19.56 and 58.16 respectively as provided by the manufacturer. The homogenized sample was filled up to half of the circular 2.5 inch glass cell and measured at three different locations. L* (lightness), a* (redness/greenness (+/-)) and b* (yellownwss/blueness (+/-)) were measured using D65 illuminant, 100 standard observer.

3.2.4 Microbiological analysis

Sample preparation

Ten gram of fish sample was aseptically weighed and homogenized with 90 ml of normal saline (0.85%) for one minute in a stomacher at 230 rpm (Seward Stomacher 400 Circular, London, UK). The homogenized sample was serially diluted using sterile 9ml saline for bacteriological analysis

3.2.4.1 Total mesophilic count

Total mesophilic counts were determined by the spread plate method using Plate count agar (PCA) (Difco) as per Townley & Lanier (1981) method. 0.5 ml of appropriate dilution was spread on the pre set sterile PCA plates in duplicate. For measuring mesophilic counts, plates were incubated at 30^0C for 48 hours. The average counts were calculated and expressed as colony forming units per gram (log $cfug^{-1}$) of the sample.

3.2.4.2 Total enterobacteriaceae

Enterobacteriaceae were enumerated on Violet Red Bile Glucose Agar (VRBGA, Oxoid, CM 485) as per Koutsoumanis & Nychas (1999). One ml of appropriate serial dilutions of fish homogenate was inoculated into 15 ml of molten VRBGA medium cooled to 45^0C mixed well and allowed to set for 15 min. The average counts were calculated and expressed as colony forming units of Enterobacteriaceae per gram (log $cfug^{-1}$) of the sample.

3.2.4.3 H_2S producing bacteria

Hydrogen sulphide producing bacteria were eneumerated on Peptone Iron Agar (PIA) (code 289100, BBL Difco) with added 1% salt (Gram et al., 1987). Appropriate dilutions of the sample (0.5ml) was added in duplicate to pre drained sterile PIA plates and spread evenly using sterile bend glass rod. After spreading, the plates were overlaid with the same medium and incubated at 20^0C for 5 days. Black colonies formed by the production of H_2S were enumerated (Gennari & Campanini, 1991) and the counts were expressed as number of colony forming units of H_2S producing bacteria per gram of the sample (log $cfug^{-1}$)

3.2.4.4 Lactic acid bacteria

Lactic acid bacteria were enumerated on MRS agar (de Man, Rogosa and Sharpe, Himedia code M 641) supplemented with potassium sorbate as per Mossel (1987). Samples (0.5ml) of appropriate dilutions were added in duplicate to pre dried sterile MRS agar plates and spread evenly using sterile bend glass rod. After spreading, plates were overlaid with the same medium and incubated at ambient temperature in an anaerobic jar for 72 hrs. Pure white colonies (2-3mm dia) were counted as *Lactobacillus spp*.

The average count was calculated and expressed as colony forming units per gram (log cfu g^{-1})

3.2.4.5 Brochothrix thermosphacta

For the eneumeration of *B thermosphacta*, Streptomycin Thallus Acetate Acetidione Agar (STAA) was used (Mead & adams, 1977). Samples (0.5ml) of appropriate serial dilution were added in duplicate to pre dried sterile STAA plates and spread evenly using sterile bend glass rod. The plates were then incubated at 20^0C for 5 days. To the incubated plates cytochrome oxidase reagent was added and the white colonies were counted as *B. themosphacta*. Counts were expressed as number of colony forming units per gram of fish (log $cfug^{-1}$).

3.2.4.6 *Faecal streptococci*

Faecal streptococci were enumerated on Kenner Faecal Agar (KF, BBL, Difco) USFDA, (1995). One ml of appropriate serial dilutions of fish homogenate was pipetted to sterile petridish taken in duplicate for each dilution. About 15-18 ml of molten KF agar cooled to 45^0C and poured to each plate. It was then mixed well and allowed to set for 30 min. The plates were then incubated at 37^0C for 48 h. All red to pink colonies were counted as *Faecal Streptococci*. The average count of duplicate was calculated and expressed as colony forming unit of *F streptococci* per gram (log $cfug^{-1}$) of the sample.

3.2.4.7 Escherichia coli

E coli was enumerated on Tergitol 7 (T7) agar. 0.5 ml of appropriate serial dilutions of fish homogenate was spread in duplicate on pre set T7 plates and allowed to dry for 15 min and incubated at 37^0C for 24 h. Colonies with lime yellow, occasionally with rust brown center and yellow

zone around were isolated and confirmed as *E coli* on EMB agar (Eosin methylene blue agar) plates and by IMViC test (Surendran et al., 2009)

3.2.4.8 Staphylococcus aureus

Staphylococcus aureus was enumerated on Baired Parker Agar (BP, Difco) (AOAC, 2000). Appropriate serial dilutions of fish homogenate (0.5 ml) were spread on pre dried sterile BP agar plates and incubated at 37^0Cfor 48 h. The black, convex colonies with clear margin zone were counted as *Staphylococcus aureus*. Typical colonies were isolated and confirmed by coagulase test. Counts were expressed as number of colony forming units of S*taphylococcus* per gram (log $cfug^{-1}$) of the sample.

3.2.4.9 Mouse bioassay for *Clostridium botulinum* toxin

C. botulinum toxin was detected by mouse bioassay as per the procedure described in US Food and Drug Administration Bacteriological Analytical Manual (USFDA, 2001). An extract of the sample was made by homogenizing the sample with a suitable volume (1:2) of gelatin phosphate buffer (pH 6.2). It was then centrifuged at 10000g for 20 min at 4^0C and the supernatant was tested for botulinum toxin by mouse bioassay. A portion of the supernatant was diluted to 1:5 using gelatin-phosphate buffer and divided in to 3 aliquots. One aliquot was treated with trypsin after adjusting the pH to 6.2 with NaOH or HCl (0.2 ml of Trypsn (Difco)1:250, 10% solution was added to 1.8 ml (diluted supernatant) and incubated at 37^0C for 1 h. Second aliquot was heated for 10 min in a boiling water bath and cooled and the third aliquot was kept untreated. 0.5 ml of each of the aliquots (untreated, trypsinised and heated supernatant) were injected separately into intra-peritonial region of the Webster mice weighing 18-20 g using a 2 ml sterile syringe with a 5/8 inch 25 gauge needle, leaving a pair of mice as control. All

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

3.2.5 Sensory analysis

Sensory analysis of raw and cooked samples were carried out by ten trained panelists using a nine point hedonic scale as prescribed by Meilgaard et al., (1999) (Annexure I). Samples were cooked in 1.5% salt solution for 10 minutes and were cooled. Samples were provided in a coded plate. The panelists were asked to score for appearance, colour, and odour for raw samples and for cooked sample attributes such as flavor, texture, and taste were used. Overall acceptability was calculated by adding all the attributes and dividing by total number of attributes. A score of above four was considered as the margin of acceptability.

3.2.6 Statistical analysis

Data are collected in triplicate and the results are presented as mean ± standard deviation. Experimental data were analyzed using two way analysis of variance. Marginal means of treatments and storage days were compared using tuky's post hoc test and least significant difference (lsd) was calculated for comparing interaction means at 5% level of significance. All the analysis were carried out using SAS 9.3 and SPSS 20.00.

Chapter -4

STANDARDIZATION OF CHEMICAL MIXTURES FOR THE DEVELOPMENT OF DUAL ACTION SACHETS WITH CO_2 EMITTER AND O_2 SCAVENGER

Contents

4.1 Selection of packaging material

In the present work, Ethylene vinyl alcohol co-polymer was used as the packaging material. EVOH is a common packaging material that is well known for its excellent oxygen barrier property (López-de-Dicastillo et al., 2012). The physical properties of packaging materials used in the study are given in Table 4.1. The EVOH used in the study showed a good tensile strength and heat seal strength. The oxygen, carbon dioxide and water vapour permeability of EVOH was found to be very less which indicates the suitability of this packaging material for active packaging studies. Overall migration of packaging material was tested with water and n heptane and the results were well below the limits specified for food contact application (FDA, 1983, IS 9845, 1981).

High molecular weight high density polyethylene (HM-HDPE) film was used for making dual action sachets with carbon dioxide emission and oxygen scavenging capacity. The physicochemical properties of HM-HDPE film used for making sachets are given in the table 4.1. The carbon

dioxide, oxygen and watervapour transmission rate of HM-HDPE was found to be very high, which indicates its suitability for making dual action sachets (Table 4.1).

Table 4.1 Physico-chemical properties of packaging material used in the study

Parameter	EVOH	HMHDPE
Thickness	0.14mm	0.015 mm
Tensile strength		
Machine direction	248.57 kg/cm^2	222.79kg/cm^2
Cross direction	243.3 kg/cm^2	199.56 kg/cm^2
Elongation at break		
Machine direction	533.32%	473.44%
Cross direction	460.20%	197.63%
Heat seal strength		
Machine direction	160.95kg/cm^2	241 kg/cm^2
Cross direction	152.38kg/cm^2	195.95 kg/cm^2
Water vapour transmition rate	1.7g/m^2/24hr at 92%RH and 38C%	14.81g/m^2/24hr at 92%RH and 38C%
Carbon dioxide transmission rate	96.18cc/m^2/24hr	1312 cc/m^2/24hr
Oxygen transmission rate	60.8cc/m^2/24hr	756 cc/m^2/24hr
Overall migration Mg/L		
Water extractives	20.5	-
n-heptane	10.3	-

4.2 Standardization of chemical mixtures

Eighteen different chemical combinations were used to develop dual action scavengers with oxygen scavenging and carbon dioxide emitting capacity. The different combinations tried are given in Table 4.2. The dual action sachets were tried with the principle that when bicarbonate compounds react with acids, carbon dioxide is released along with the formation of other

compounds. In the present study, iron powder and ascorbic acid were used for oxygen scavenging, where ascorbic acid accelerates the scavenging action. Sodium bicarbonate was used as a medium for carbon dioxide emission. The iron powder under appropriate humidity conditions, uses up residual oxygen to form non toxic iron oxide. The oxidation mechanism can be expressd as follows (Smith et al., 1990; Smith, 1992)

$Fe \rightarrow Fe^{2+} + 2e$

$½\ O_2 + H_2O + 2e \rightarrow 2OH^-$

$Fe^{2+} + 2(OH)^- \rightarrow Fe(OH)_2$

$Fe(OH)_2 + ½\ O_2 + ½\ H_2O \rightarrow Fe(OH_3)$

The appropriate size of the scavenger /emitter was calculated by using the method developed by Roussel (1999) and ATCO technical information (2002).

$$A = (V-P) \times [CO_2]/100$$

Where V = volume of finished pack determined by submersion in water (ml)

P= weight of the finished pack (g)

$[CO_2]$ = initial CO_2 concentration in package

In addition, the volume of CO_2 (ml) likely to permeate through the packaging during shelf life of the product (B) was calculated as

$B = S \times P \times D$

Where S = surface area of the pack (m^2)

P = permeability of the packaging (ml m^{-2} 24 h^{-1} atm^{-1})

D = shelf life of the product (days)

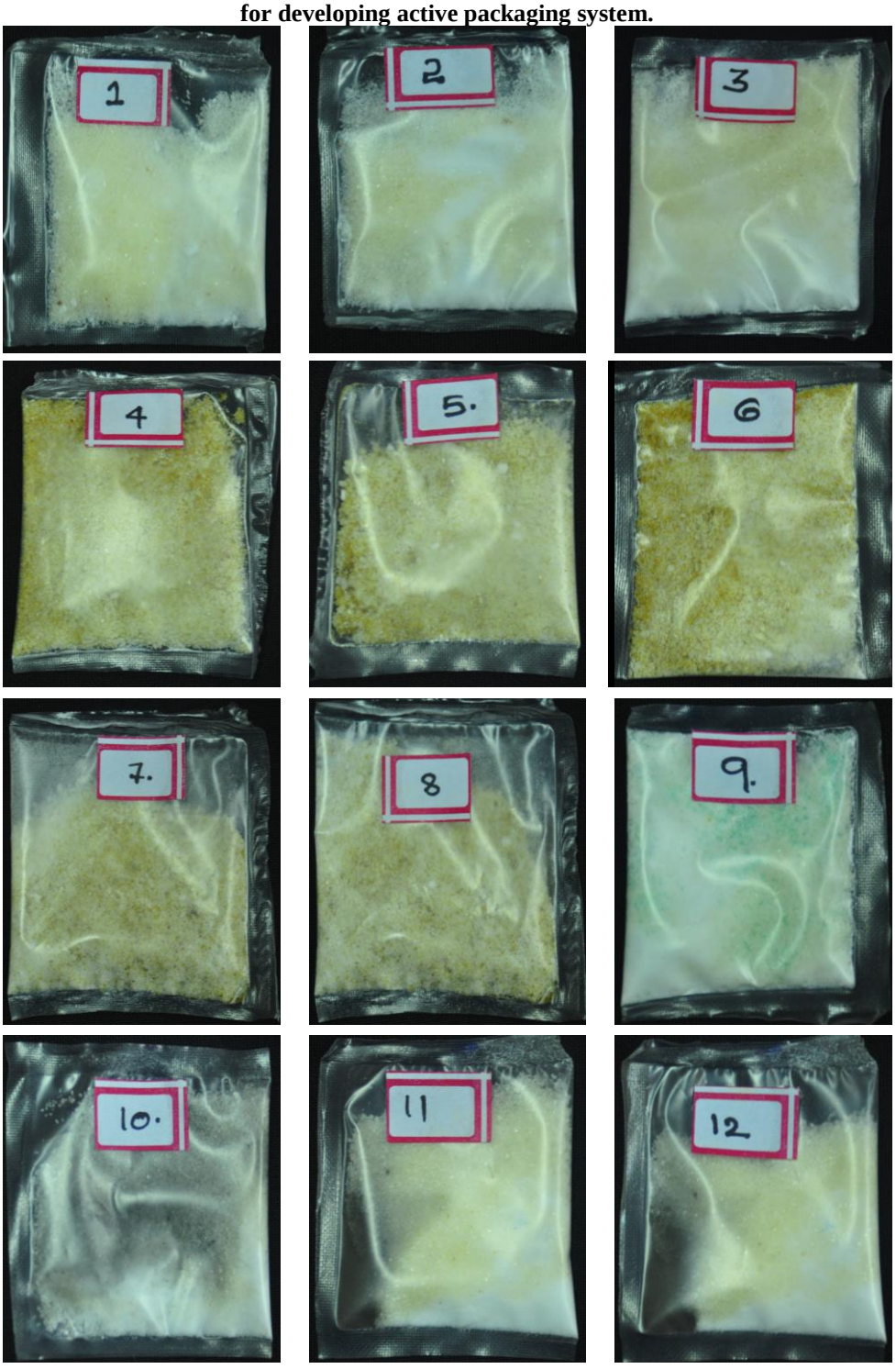
The volume of CO_2 to be emitted was obtained by subtracting A from B. As a rule of thumb, 1g iron powder is sufficient to scavenge 300ml of O_2 present in the pack. Based on this calculation, the number of sachets required for the emission or absorption of gases was determined. The amount of chemicals

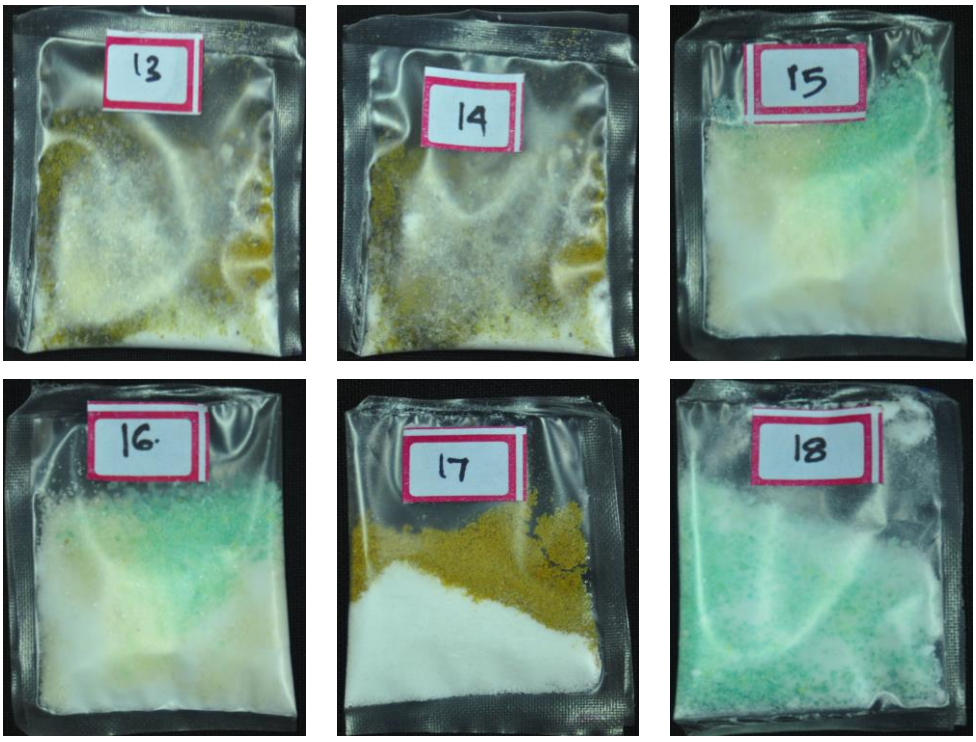
used in each sachet for emitting CO_2 and for absorbing O_2 is given in the Table 4.2.

Table : 4.2. Chemical combinations used for developing active packaging system

Sl No (code)	Chemical combinations
1	1g sodium bicarbonate + 2g ascorbic acid
2	1 g sodium bicarbonate + 1 g ascorbic acid
3	2g sodium bicarbonate + 2 g ascorbic acid
4	1g sodium bicarbonate + 3 g ascorbic acid + I g EDTA iron powder
5	1g sodium bicarbonate + 2g ascorbic acid + 1g EDTA iron powder
6	1g sodium bicarbonate + 1g ascorbic acid + 1 g EDTA iron powder
7	3g sodium bicarbonate + 1g ascorbic acid + 1g EDTA iron powder + 1g ferric carbonate
8	2g sodium bicarbonate +1g ascorbic acid+ 1g EDTA iron powder + 1g ferric carbonate
9	2g sodium bicarbonate + 1g ascorbic acid +1g ferric sulphate
10	1g sodium bicarbonate+3g ascorbic acid + 1g iron powder
11	1g sodium bicarbonate + 2g ascorbic acid + 1g iron powder
12	2g sodium bicarbonate+ 2g ascorbic acid +1g iron powder
13	1g sodium bicarbonate +3g ascorbic acid +1g iron powder + 1g ferric carbonate
14	1g sodium bicarbonate+2g ascorbic acid + 1g iron powder + 1g ferric carbonate
15	2g sodium bicarbonate + 2g ascorbic acid + 1g iron powder + 1g ferric carbonate
16	2g sodium bicarbonate+ 2g ascorbic acid + 1g ferrous sulphate
17	2g sodium bisulphate + 2g ferric carbonate
18	2g sodium bisulphate + 2g ferric sulphate

Plate 4.1 Chemical combinations in HMHDPE sachets used for developing active packaging system.





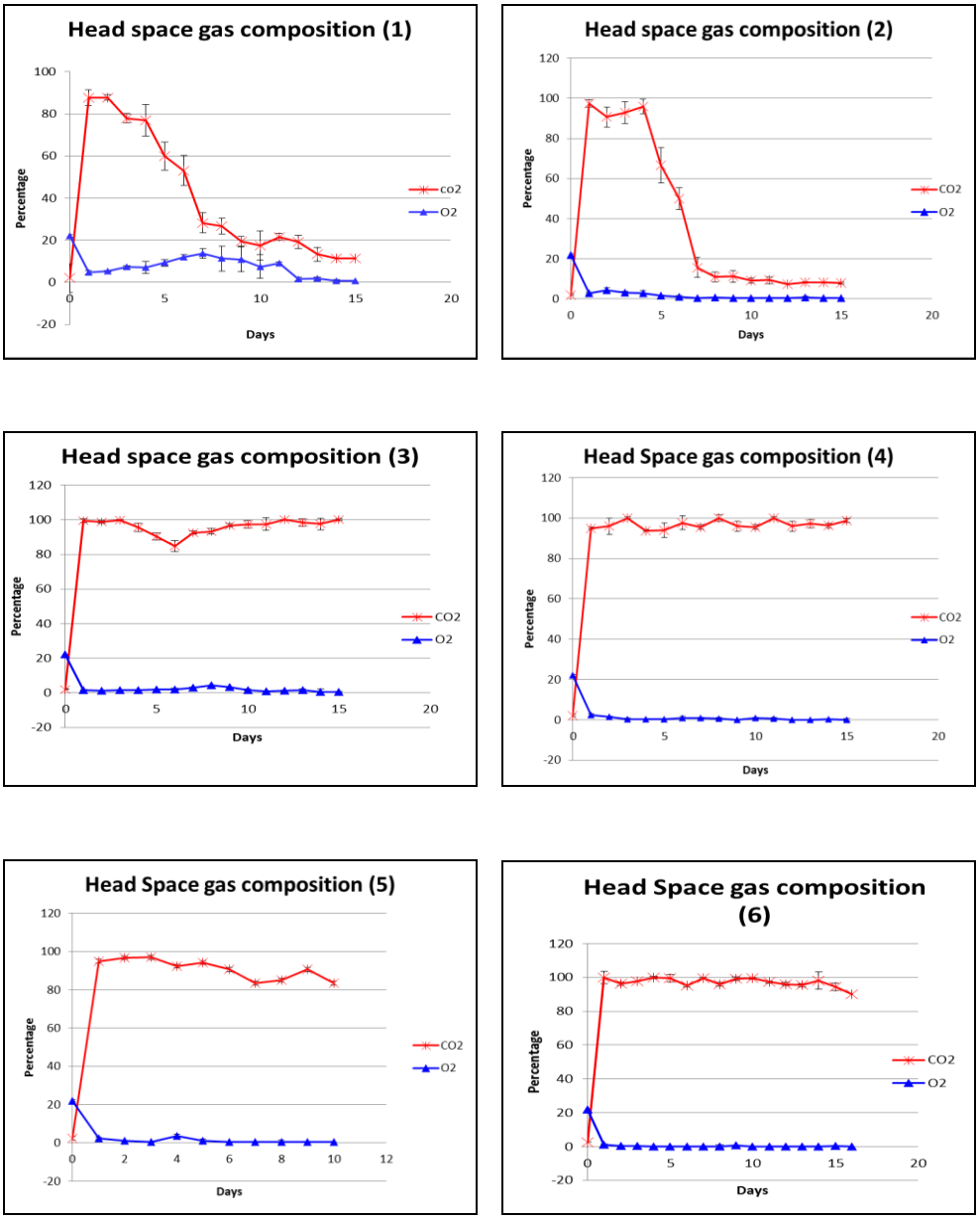
The developed sachets were tested for the liberation of CO_2 and for the absorption of O_2 quantitatively by using gas analyzer (PBI Dansensor, Checkmate 9900, Ronnedevej, Denmark). The developed sachets (5 x 5 cm) were packed in EVOH pouches (13 x 15 cm) along with 2 ml of water. The packs were sealed with a sealing machine (Model No 405, Samarpan Fabarications Pvt Ltd, thane, India) and kept at room temperature for further analysis. Five packs from each batch were tested for head space gas composition to analyse the production of CO_2 and the removal of oxygen inside the package. The head space gas composition of each packs stored at room temperature is given in the Fig. 4.1

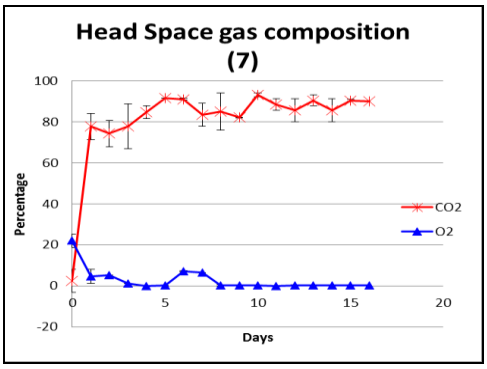
The initial CO_2 and O_2 concentartion inside the pack was 2% and 22% respectively. The first (code 1) chemical combination reached above 85% CO_2 within 24 hours but the concentration reduced on storage. In addition, the oxygen concentration did not even reduce to below 0.5% until

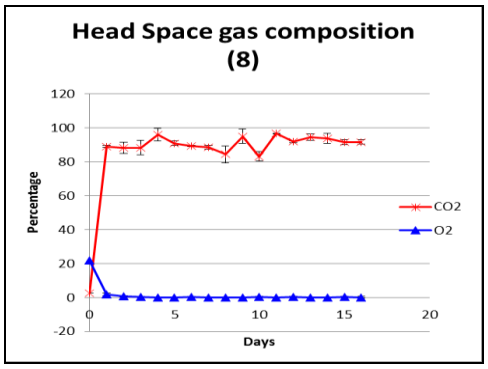
the end of storage. The CO_2 concentration of 2nd chemical combination reached above 75% on the second day of storage and the O_2 concentration reduced to 0.5% on 7th day of storage. Both the 1st and 2nd chemical combinations did not maintain the CO_2 concentartion during storage. However the other chemical combinations from 3rd to 16th maintained the increased CO_2 concentartion till the end of storage period. The 12th, 14th and 16th combinations reduced the O_2 concentartion inside the packages to less than 0.7, 0.5 and 0.8% within 24 hours of storage respectively. It was then further reduced to less than 0.03, 0.008, and 0.03 on 7th, 3rd and 6th day of storage respectively. The samples then reached 0% oxygen concentration and maintained the oxygen free atmosphere till the end of storage along with higher CO_2 concentration. The samples packed with 17 and 18 number combinations were effective in reducing oxygen content inside the package headspace. But both 17 and 18 number chemical combinations were not effective to reduce the O_2 content to less than 1% during storage time. The CO_2 concentration did not increase during storage in these two chemical combinations. Moreover the packaging material showed some deformation during storage. Hence the combinations 17 and 18 were rejected without further analysis.

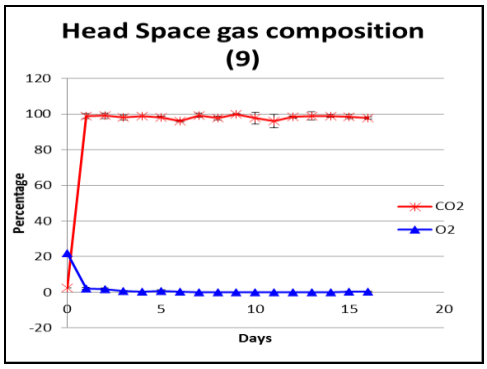
The above mentioned chemical combinations were tried with chilled storage for analyzing the gas composition inside the package head space. The chemical combinations were effective in reducing the O_2 content inside the packs within 48 hours of storage. All packs showed an increasing trend in CO_2 formation inside the package within 24 hours of storage. But the packs collapsed upon further storage and did not show the same CO_2 concentration with respect to the counterparts at room temperature storage. This may be due to the dissolution of CO_2 during chilled storage since the solubility of CO_2 increase with decrease in temperature.

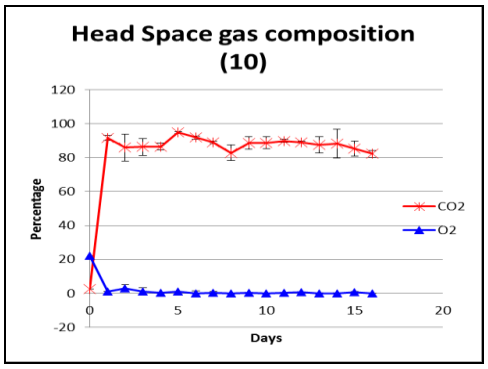
Fig. 4.1. Changes in the head space gas composition, CO_2 and O_2 (%) by different chemical combinations used for developing active packing system. (Numbers represent each chemical combinations represented in table 4.2.) Mean ±standard deviation, n = 5.

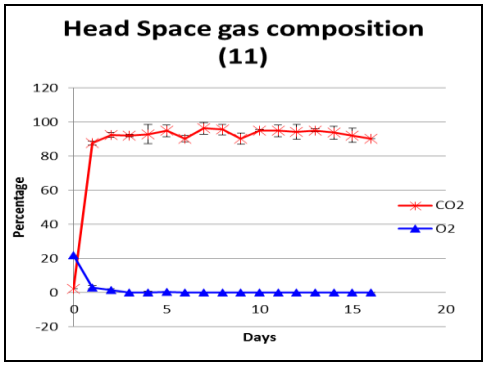


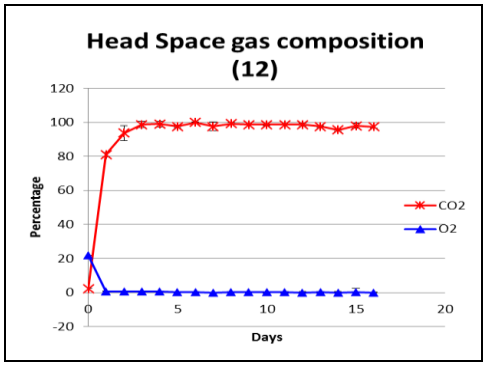

Head Space gas composition (7)
Percentage
Days
CO2
O2

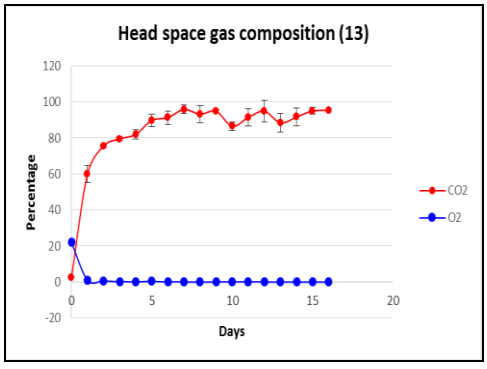

Head Space gas composition (8)
Percentage
Days
CO2
O2

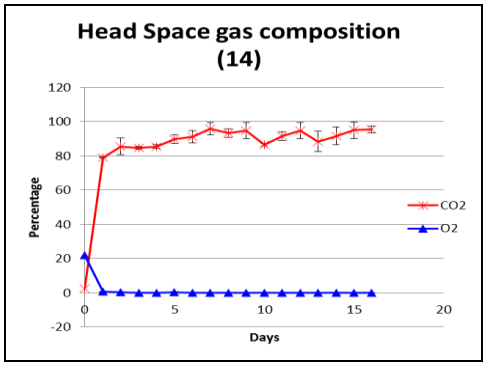

Head Space gas composition (9)
Percentage
Days
CO2
O2

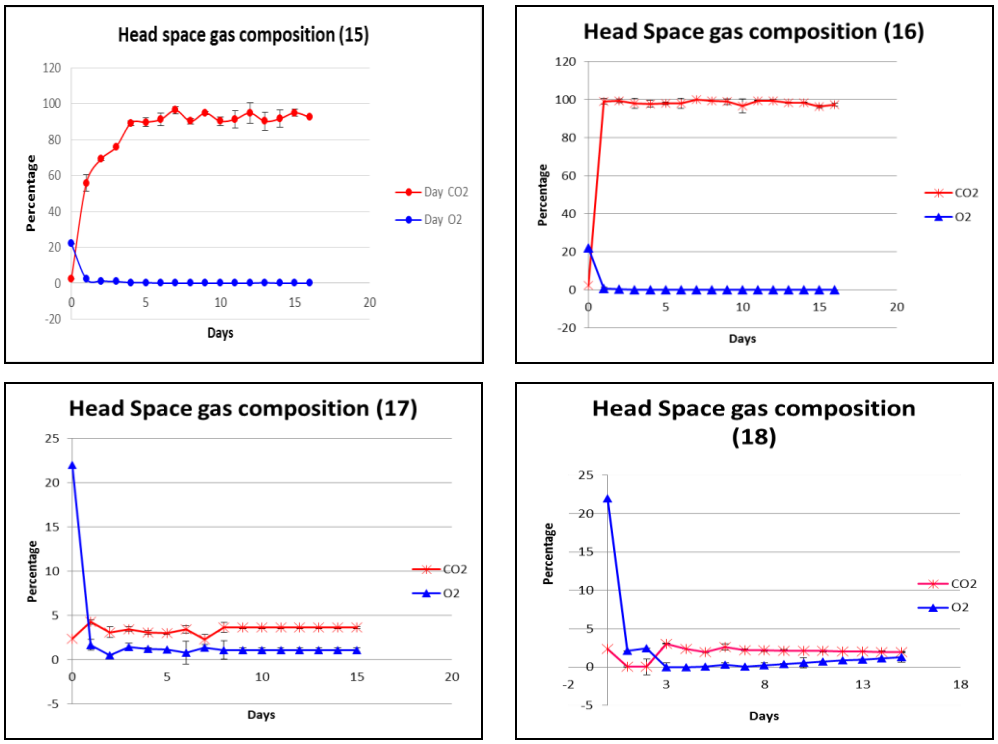

Head Space gas composition (10)
Percentage
Days
CO2
O2


Head Space gas composition (11)
Percentage
Days
CO2
O2


Head Space gas composition (12)
Percentage
Days
CO2
O2


Head space gas composition (13)
Percentage
Days
CO2
O2


Head Space gas composition (14)
Percentage
Days
CO2
O2



4.3 Order of the chemical reaction and rate constant

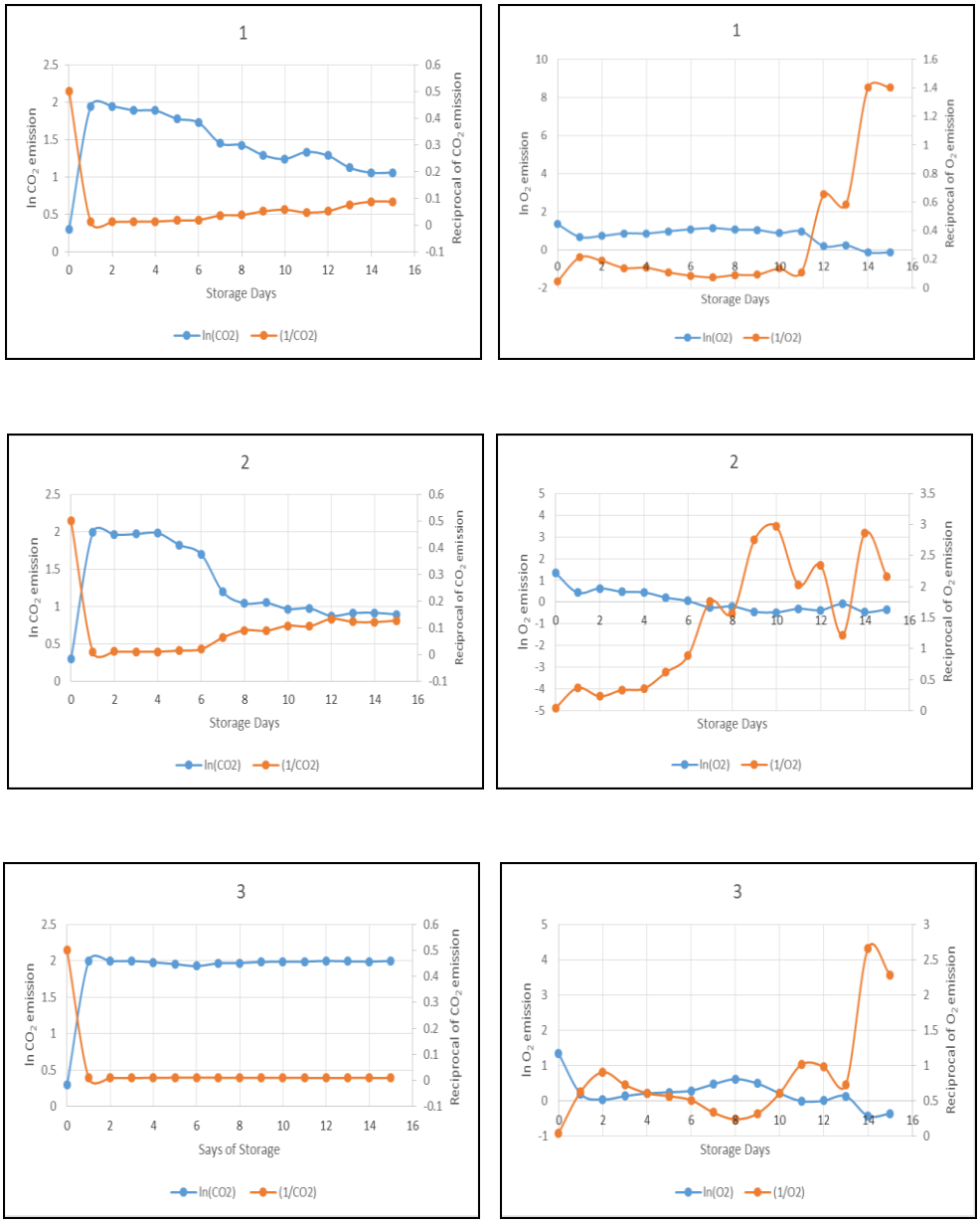
Order of the reaction was determined by plotting the natural logarithm (ln) for CO_2 emission (or O_2 absorption) and the reciprocals of the volumes of CO_2 emitted (or O_2 absorbed) in the pack against time. If the natural log plot approximated a straight line, the reaction was regarded as first order. If the reciprocal plot approximated a straight line, the reaction was regarded as second order. Rate constant were calculated using the following equations (Brown et al., 1994)

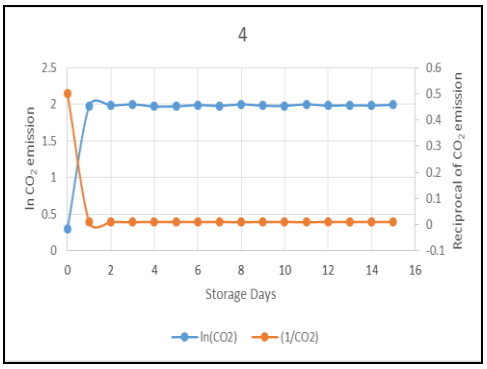
For first order reaction: $\ln[A]_t = kt + (1/[A]_0)$

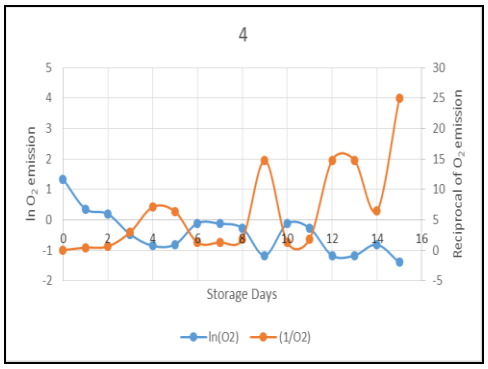
For second order reaction $1/[A]_t = kt + (1/[A]_0)$

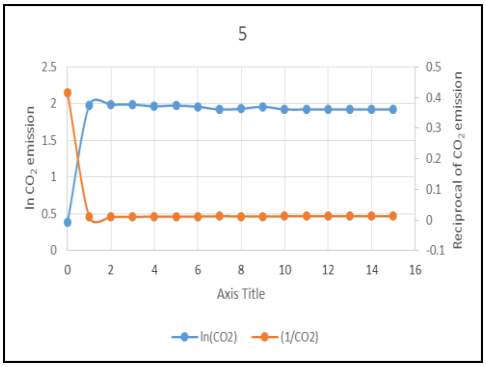
Where $[A]_t$ was the amount of reactant A (CO_2 or O_2) at the time 't' (h), 'k' is the rate constant and $[A]_0$ is the initial amount of reactant (CO_2 or O_2).

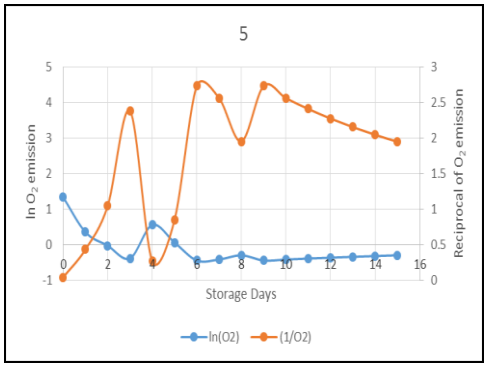
Fig. 4.2. Plot of Natural logarithms (ln) vs reciprocal volume CO_2/O_2 remaining inside the packs by using different chemical combinations.

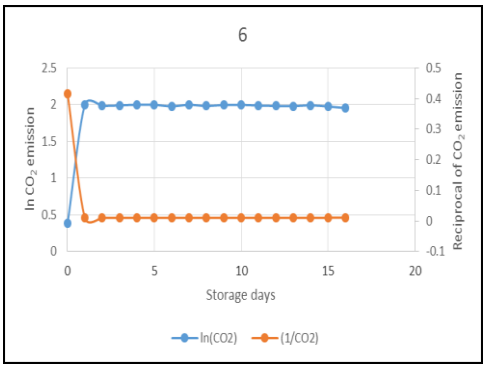


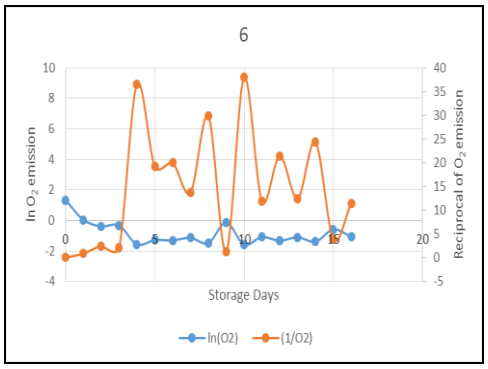

4
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)

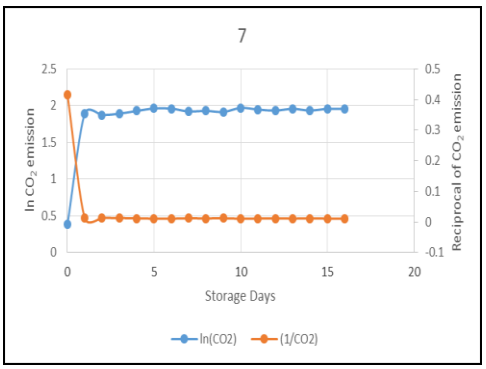

4
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)


5
ln CO2 emission
Reciprocal of CO2 emission
Axis Title
ln(CO2)
(1/CO2)


5
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)


6
ln CO2 emission
Reciprocal of CO2 emission
Storage days
ln(CO2)
(1/CO2)


6
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)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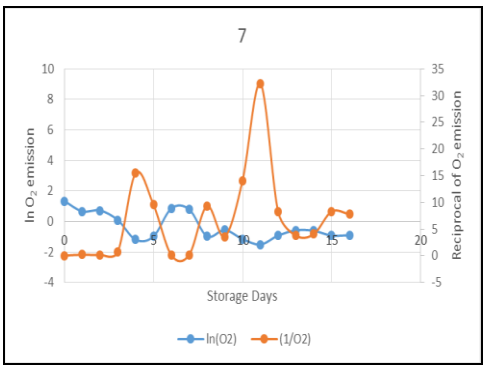
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)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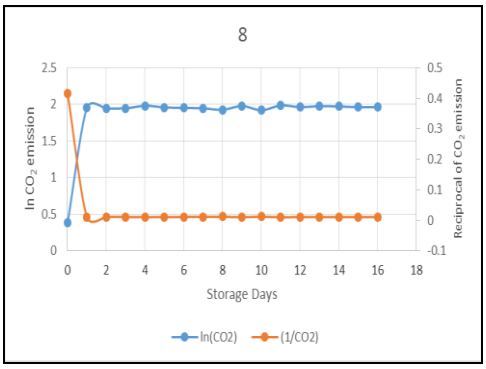
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)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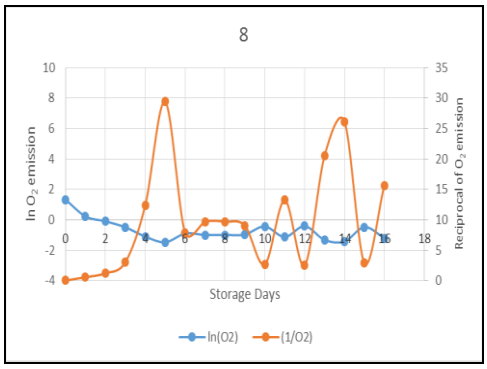
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)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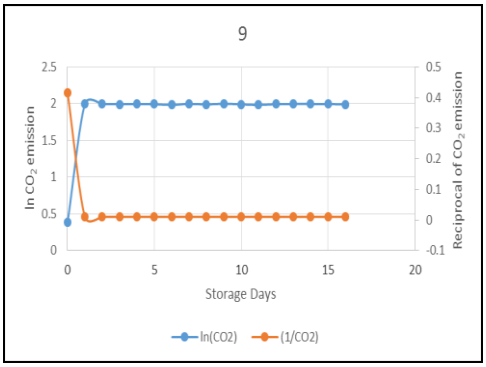
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)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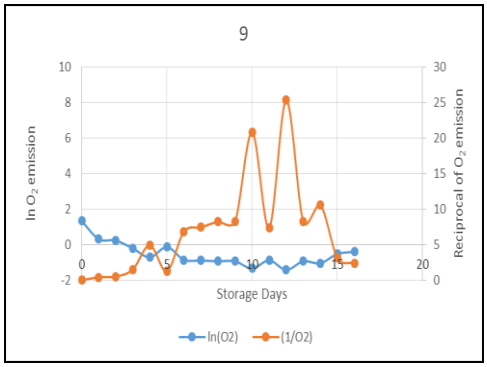
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)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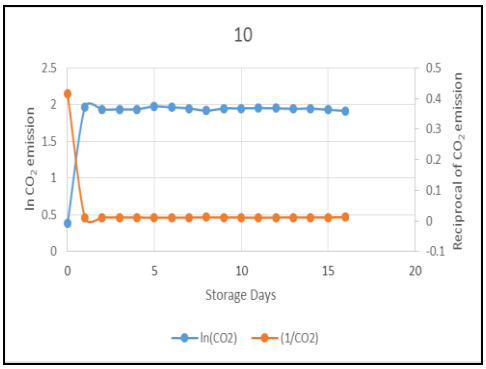
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)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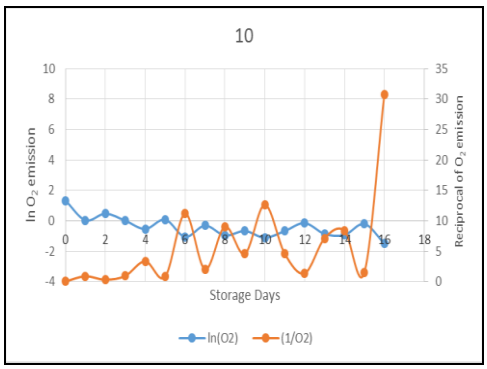
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)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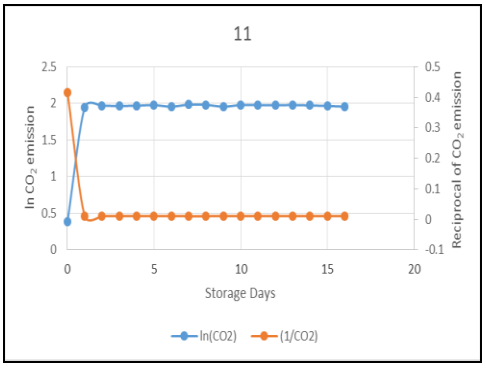
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)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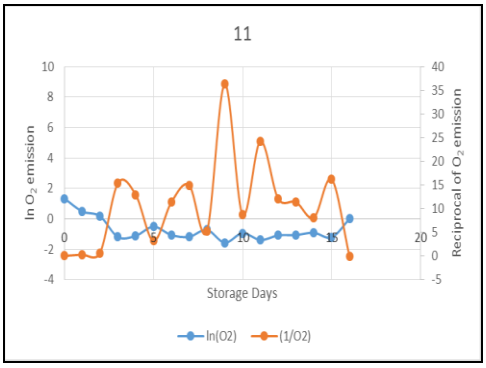
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)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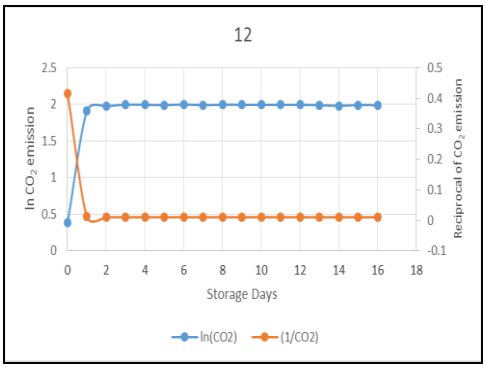
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)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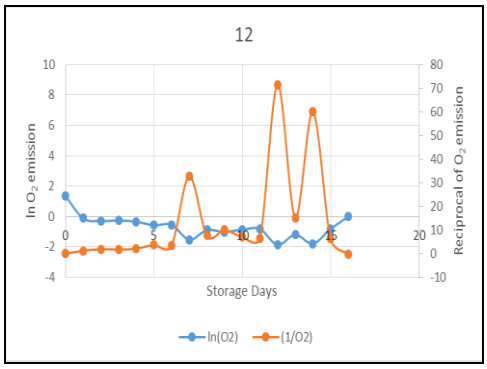
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)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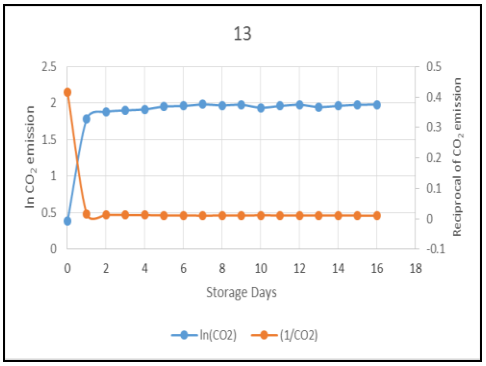
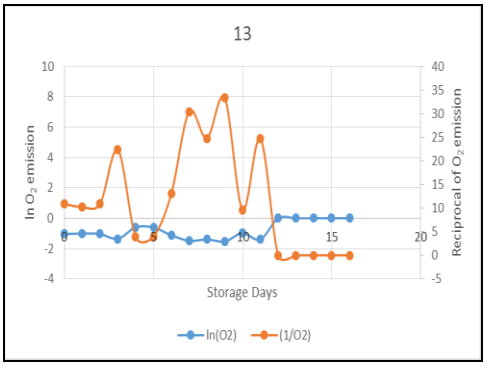
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)


13
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)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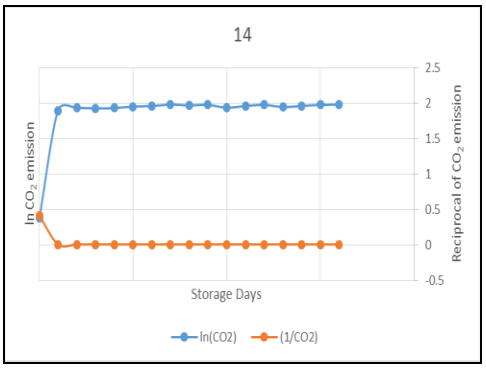
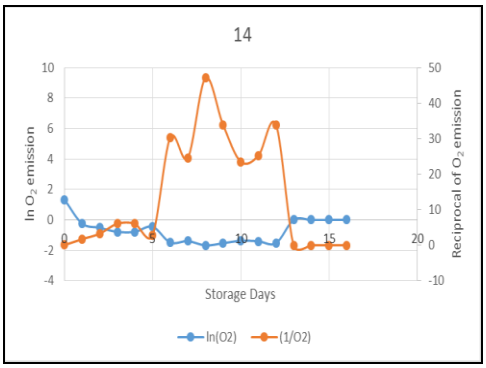
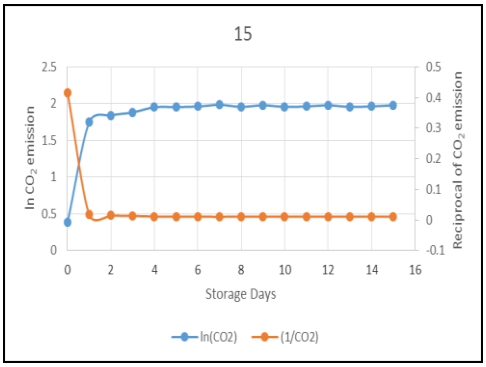
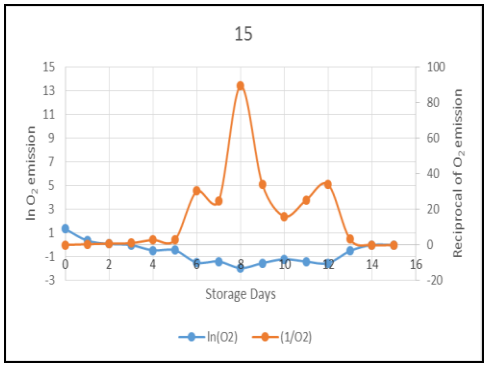
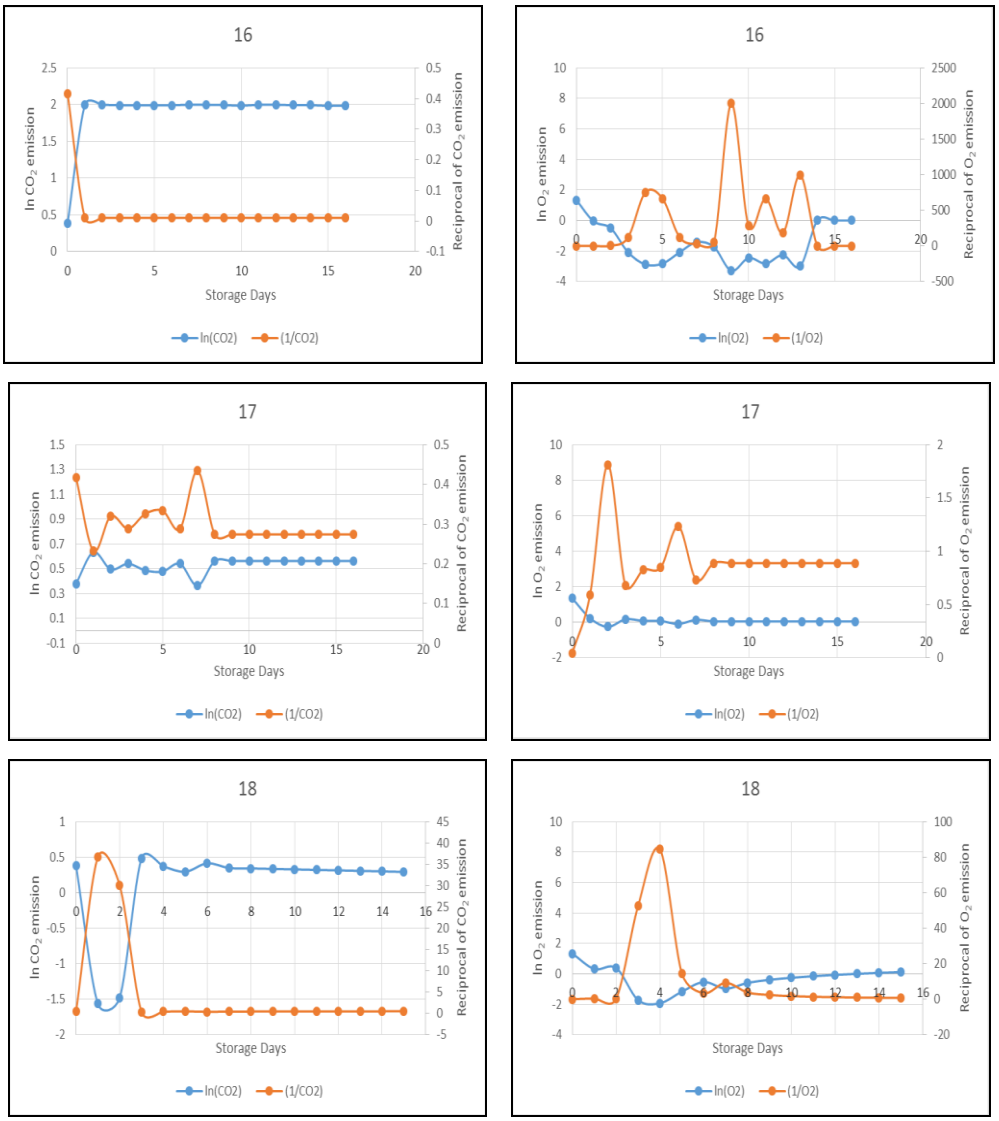
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)


14
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)


14
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)


15
ln CO2 emission
Reciprocal of CO2 emission
Storage Days
ln(CO2)
(1/CO2)


15
ln O2 emission
Reciprocal of O2 emission
Storage Days
ln(O2)
(1/O2)



From the present study, the CO_2 emission and O_2 absorption reaction of the chemical combinations of developed sachets followed first order reaction (Fig 4.2). The rate constants were calculated from the first order kinetics equation and were generally higher values were observed for CO_2 emission compared to O_2 absorption (Table 4.3.). Considerable variability in the CO_2 emission and O_2 absorption capacity was observed in different chemical combinations mainly due to the differences in the reaction

constants. This could be due to the non uniform size of chemicals, and imperfect mixing (Tewari et al., 2002; Mohan, 2008; Gill & McGinnis, 1995).

Table 4.3 Rate constant (k) of first order kinetics equation.

Sample code	CO_2	O_2
1	0.921±0.43	0.685±0.45
2	0.783±0.53	-0.0024
3	1.377±0.42	0.182
4	1.38±0.42	0.1819
5	1.437±0.39	0.1522
6	1.482±0.38	-0.887
7	1.425±0.37	-0.385
8	1.454±0.38	-0.735
9	1.487±0.391	-0.577
10	1.43±0.37	-0.451
11	1.458±0.38	-0.788
12	1.481±0.38	-0.76
13	1.43±0.45	-1.33
14	1.452±0.38	-0.952
15	1.42±0.39	-0.76
16	1.488±0.39	-1.9073
17	0.1179±0.06	0.08083
18	-0.2972	-0.388

Mean ± standard deviation. n = 5.

4.4 Selection of dual action sachets for further studies

In the present study, inorder to select the best three combinations for further studies, two way analysis of variance was performed to analyse the carbon dioxide emission and oxygen scavenging rate of developed chemical combinations with respect to storage days. The marginal means of treatment and storage days were computed and compared by Tukey's test to select an appropriate treatment. A desirability score was computed and cross

checked with the Tukey's test before selecting the treatments (Table 4.4; Fig 4.3.). All the analysis were done by using a code in SAS 9.3. It was observed that the developed chemical combination with 12, 14 and 16 combination exhibited the best dual action of CO_2 emission along with O_2 scavenging in the package headspace throughout the storage days. Based on the above results, the combinations 12, 14 and 16 were chosen for further studies as the CO_2 emission capacity was above 80% in all the three combinations apart from their ability to scavenge O_2 very rapidly from the head space.

Table 4.4 Tukey's studentized range (HSD) test for CO_2 and O_2

Treatment	CO_2	O_2
1	39.18^{H}	7.90^{A}
2	39.17^{H}	2.98^{CB}
3	80.88^{FDE}	3.47^{B}
4	84.81^{B}	2.16^{EDF}
5	78.65^{F}	2.45^{CD}
6	91.43^{A}	1.62^{GHF}
7	80.54^{FE}	2.66^{CD}
8	84.16^{B}	1.79^{EGHF}
9	89.81^{A}	2.12^{EGDF}
10	82.29^{DE}	2.15^{EDF}
11	86.74^{B}	1.84^{EGHF}
12	90.82^{A}	1.61^{GHF}
13	75.43^{G}	2.31^{ED}
14	83.65^{D}	1.52^{H}
15	74.59^{G}	2.51^{CD}
16	91.38^{A}	1.57^{GH}
17	3.07^{I}	2.51^{CD}
18	2.23^{I}	2.11^{EGDF}

Mean with the same lettetr are not significantly different. Mean ± standard deviation, n = 5

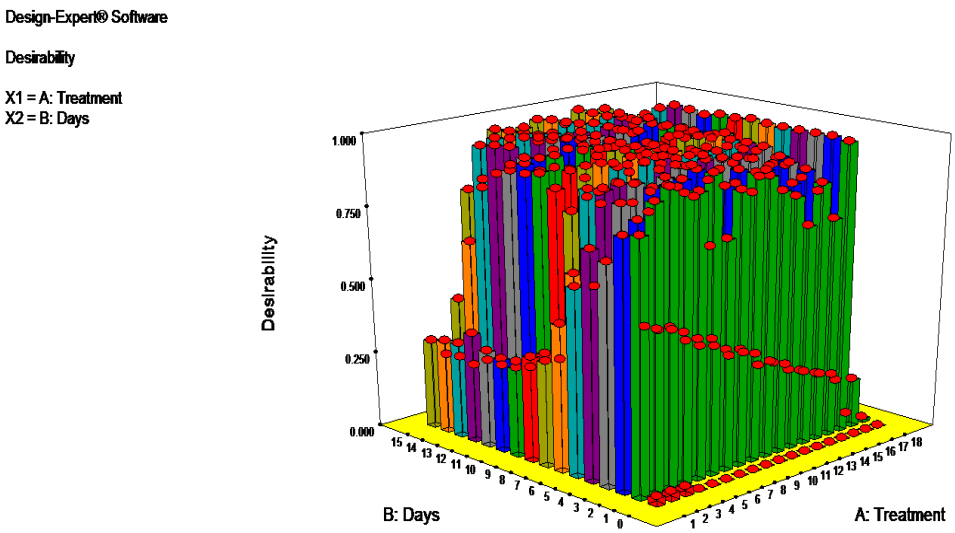


Fig. 4.3. Desirability score of various chemical combinations used for developing dual action sachets. Mean ± standard deviation, n = 5

Chapter -5
QUALITY CHARACTERISTICS OF YELLOWFIN TUNA (*Thunnus albacares* Bonnaterre, 1788)

Content

Abstract

Yellowfin tuna is one of the commercially important fish species and the quality characteristics of yellowfin tuna were investigated. Yellowfin tuna muscle had 23.18% crude protein and 1.52% crude fat. Tuna protein was well balanced with amino acids and the percentage ratio of essential amino acids to total amino acids was 48.2. Tuna meat was rich in docosahexaenoic acid and eicosapentaenoic acid. Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid contents of raw tuna were 31.19%, 8.23% and 58.79%, respectively. The n-3/n-6 polyunsaturated fatty acid ratio was 6.78, showing that yellowfin tuna meat is rich in n-3 PUFA. The other quality parameters like total volatile base nitrogen and trimethyl amine were well within acceptable limits. Primary and secondary lipid oxidation products were also studied. The surface colour parameters and texture of yellowfin tuna meat showed that the samples used in the present study were of good quality.

5.1. Introduction

Tuna is one of the commercially important fish species belonging to the family Scombridae (FAO, 2016) . They are commercially important in many countries and there is a high demand in the international markets. Among tuna, yellowfin tuna is an important species in the global commercial tuna fishery (FOC, 2016) contributing about 26% to the 2013 catch of principal tunas (FAO, 2016). Large quantities of yellowfin tuna are commercially used as canned, dry and salted products like cured tuna loin and sashimi, a delicious raw fish product famous in Japanese and Korean markets. Because of the highly perishable nature of fish, the success of seafood industry depends on getting the product to the consumer in acceptable condition since the consumers prefer fresh fish (De Silva & DAM, 2011). The term quality refers to the sensory characteristics of the product including appearance, flavor, odour and texture. Quality also indicates the nutritional value and safety of the product which determines the acquiring behavior of consumer (Jones & Disney, 1996). Considering the nutritional benefits associated with fish consumption an attempt was made to characterize the quality of yellowfin tuna generally available in the local markets of Cochin, India.

5.2. Materials and methods

Yellow fin tuna was procured from a fish landing center at Fort Cochin, Kerala, India. The average weight of fish used in the study was 7.5±1.4 kg with 71±3.8 cm length and 20±1.4 cm width. Three fish samples were purchased for the analysis and brought to the laboratory in iced condition. After reaching the laboratory, fish were washed in chilled potable water and kept in iced condition during dressing. Boneless meat used for the

analysis were collected from the dorsal side of fish. The proximate composition of the samples was determined by AOAC (2000) method. Amino acid concentration (Ishida et al., 1981) Tryptophan (Sastry & Tummuru, 1985) and fatty acid composition were estimated as described by AOCS (1989) method. The minerals like sodium, potassium, calcium and iron were analyzed according to APHA (1998) using flame photometer (BWB Technologies, United Kingdom). Cholesterol content was estimated by Zlatkis et al. (1953) method and expressed as $mg100g^{-1}$ of tissue. pH was measured according to APHA (1998) using a digital pH meter (Cyberscan 510, Eutech instruments, Singapore). TVB-N and TMA were estimated by the micro diffusion method (Conway, 1962). Thiobarbituric acid value of the sample was estimated spectrophotometrically (Tarladgis et al., 1960) and expressed as mg malonaldehyde kg^{-1} of sample. Free fatty acid was measured and expressed as mg % oleic acid (AOCS, 1989). Peroxide value was analysed and expressed as milli equivalent of O_2 kg^{-1} fat (AOCS, 1989). The colour of the sample was measured with Hunter colorimeter (Hunter Lab colorimeter, MiniScan XE Plus Hunter Associates Lab inc., Reston, Virginia, USA).

Texture profile analysis was measured with a Universal Testing Machine (Lloyd instruments LRX plus, UK) with a cylindrical probe of 50 mm diameter equipped with a sensor of 50N. Sensory analysis was carried out by using a 9 point hedonic scale (annexure 1) as prescribed by Meilgaard et al. (1999). All analyses were carried out in triplicate. The results were expressed as mean values ± standard deviation.

5.3. Results and discussion

5.3.1. Proximate composition of yellowfin tuna

Chemical composition is an important aspect of fish quality and it influences the quality and safety of fish (Huss, 1988). In fish, the major constituents include water, protein, fat and ash. In the present study, proximate composition of yellowfin tuna showed 73.28% moisture, 1.52% crude fat, 23.18% crude protein and 1.52% ash (Table 5.1). The proximate composition of fish varies widely between species and within the species, it varies depending on age, sex, environment, and season. According to Venugopal (2006), the protein content of most raw fin fish flesh ranged between 17-22% and the protein content of most tuna species ranges between 15-30%. The protein content of yellowfin tuna in the present study was found to be higher than other species like *Pampus punctatissimus* (Zhao et al., 2010) and seer fish (Mohan, 2008). Peng et al. (2013) observed 23.52% and 23.72% crude protein in yellow fin tuna and bigeye tuna respectively. However Nakamura et al. (2007) observed 26% protein content in bluefin tuna.

Fat content of yellowfin tuna in the present study was 1.52%, which could be classified as semi fatty fish (Özogul & Özogul, 2007). The cholesterol content of fresh tuna was found to be 31.11 mg/100g (Table 1). The cholesterol content of yellowfin tuna was found to be within the range of 30-60 mg/100g as described by Robert & Marcus (1990) in marine fish samples. Seafood is considered as a rich source of mineral components. The mineral composition of marine fish is in the range of 0.6-1.5% wet weight (Sikorski et al., 1990a). Table 5.1 shows the mineral composition of yellowfin tuna meat. The most abundant mineral was potassium followed by sodium. The average calcium and iron content in yellowfin tuna meat was 381.8 ppm and 101.86 ppm respectively. The concentration of minerals in fish muscle is influenced by the ability of fish muscle to absorb the inorganic

elements from their diet and from the water bodies they live (Adewoye & Omotosho, 1997).

Table 5.1 Proximate and mineral composition of yellowfin tuna.

Moisture	73.28±0.51%
Fat	1.52±0.12%
Protein	23.18±0.23%
Ash	1.52±0.03%
Cholesterol (mg/100g)	31.11±0.01
Sodium(ppm)	884.78±0.01
Potassium(ppm)	1500.23±0.02
Calcium(ppm)	381.80±0.01
Iron(ppm)	101.86±0.01

5.3.2. Amino acid Profile

The amino acid composition of yellowfin tuna is given in table 5.2. The most predominant essential amino acids were lysine and leucine, while tryptophan was the essential amino acid with the lowest concentration. Glutamic acid constituted the highest non-essential amino acid. Glutamic acid is known to contribute taste with degradation particles of nucleotides like inosine (Olafsdottir & Jonsdottir, 2010). The higher content of glutamic acid in the muscle makes it into more palatable during winter season (Venugopal, 2009). Lysine, a limiting amino acid in cereal based diets in developing countries was also found in significant amount in yellowfin tuna protein. Hence it can serve as a source of fortification of cereal weaning foods. 5.355 g/100 g of arginine, an essential amino acid for children was observed in present study. The percentage ratio of total essential amino acids to total amino acids was 48.2% (Table 5.3.) which is well above the level

(39%) considered to be the adequate for ideal protein food for infants, 26% for children and 11% for adults (WHO, 1985). Most of the animal protein contains less amount of cysteine compared to vegetable proteins. Cysteine is one of the sulphur containing amino acid having positive effects on mineral absorption, particularly zinc. In the present study, Cysteine/total sulphur containing amino acid (TSAA) was 26.13%. Adeyeye (2009) observed 23.8%, 28.4% and 30.1% Cys/TSAA in *C anguillaris, O niloticus* and *C senegalensis* respectively. From the present study, it was observed that the protein in yellowfin tuna muscle was found to be well balanced with essential amino acid composition and of high quality.

Table 5.2 Amino acid composition of yellowfin tuna.

Name of amino acids	g amino acid/100g of edible portion)
Essential amino acids	
Valine	4.7037±0.05
Leucine	6.9629±0.04
Threonine	3.1932±0.09
Lysine	9.589±0.06
Methionine	3.1980±0.06
Phenylalanine	3.1425±0.17
Arginine	5.355±0.12
Tyrosine	4.4862±0.54
Histidine	5.5187±0.10
Tryptophan	1.1041±0.09
Non-essential amino acids	
Glycine	3.3±0.05
Alanine	5.9424±0.02
Cysteine	1.1317±0.20
Serine	5.8163±0.54
Aspartic acid	8.127±0.12
Glutamic acid	22.1766±0.20
Proline	4.6235±0.09

(All values are the means ±standard deviations of three replicates)

Table 5.3 Concentrations of essential, non essential, acidic, basic, neutral, aliphatic, aromatic and sulphur containing amino acids (based on g amino acid/100g of edible portion).

Total amino acid (TAA)	98.37
total essential amino acid (TEAA)	47.25
total non-essential amino acid (TNEAA)	51.12
total acidic AA	30.3
total basic AA	20.46
total aliphatic AA	20.81
total aromatic AA	18.73
total neutral AA	14.62
total sulphur containing AA (TSAA)	4.32
percentage of TEAA	48.2
percentage of TNEAA	51.97
percentage of total acidic AA	30.63
percentage of basic AA	20.8
percentage of aliphatic AA	21.15
percentage of aromatic AA	19.04
percentage of neutral AA	14.86
percentage of cys in sulphur containing AA	26.13

5.3.3. Fatty acid composition

Table 5.4 presents the fatty acid composition of raw yellowfin tuna meat. Fish lipids have gained more importance because of the presence of health beneficial omega-3 polyunsaturated fatty acids (ω-3 PUFA). PUFA especially eicosapentaenoic acid and docosahexaenoic acid play a crucial role in the prevention of atherosclerosis, heart attack, depression, stroke and are believed to improve the vision and memory (Chin & Dart, 1995).

Recognizing the health benefits of ω-3 fatty acids and the serious consequences of their deficiency, the US National Institute of Health recommended a daily intake of 650 mg of ω-3 fatty acids in the form of fish (Venugopal, 2006). In the present study it was found that yellowfin tuna meat was rich in DHA and EPA (45.14% and 5.51% respectively). The DHA value was found to be higher than those reported for big eye tuna (Peng et al., 2013) and blue fin tuna (Nakamura et al., 2007). In the present study, saturated fatty acid, mono unsaturated fatty acid and PUFA contents of raw tuna were 31.19%, 8.23% and 58.79%, respectively (Table 5.4.). Arachidonic acid (C20:4) a precursor to prostaglandins and thromboxanes was also found in yellowfin tuna meat (5.35%). The major SFA were palmitic (C16:0) and stearic (C18:0) acid. The C16:0 has been reported as the major fatty acid in marine fish followed by C18:0 by Bhuiyan et al. (1986). The major MUFA's were palmitoleic (C16:1) and oleic (C18:1) acid. Another major MUFA noticed in this study was Heptadecaenoic acid. Presence of linoleic (C18:3) acid (0.31%) was also noticed in the study.

The n-3 and n-6 PUFA is have positive effects on cardiovascular diseases and certain types of cancer (Peng et al., 2013). The n-3/n-6 ratio is an important indicator for comparing the relative nutritional values of fish oils. In the present study n-3/n-6 ratio were 6.78, which was greater than the values reported by Peng et al. (2013) for two tuna species. The ratio of n-6/n-3 in the present study was 0.14, which was lower than the maximum value recommended by the UK Department of Health (HMSO, 1994). Values higher than the maximum are harmful to health and may promote cardiovascular diseases. The present study shows that yellowfin tuna meat is a good source of PUFA.

Table 5.4 Fatty acid profile of yellowfin tuna.

Fatty Acid	(% of fatty acid)
Mono Unsaturated Fatty Acid	
Eicosaenoic acid ($C_{20:1}$)	0.25±0.40
Palmitoleic acid ($C_{16:1}$)	2.27±0.14
Heptadecaenoic acid ($C_{17:1}$)	1.33±0.23
Oleic acid ($C_{18:1}$)	4.35±0.06
ΣMUFA	8.2
Poly Unsaturated Fatty Acid	
Decosahexaenoic acid ($C_{22:6}$) n3	45.14±0.2
Eicosapentaenoic acid ($C_{20:5}$) n3	5.51±0.37
Arachidonic acid ($C_{20:4}$) n6	5.35±0.03
Eicosadienoic acid($C_{20:2}$) n-6	0.45±0.01
Eicosatrienoic acid($C_{20:3}$) n3	0.28±0.01
Linolenic acid ($C_{18:3}$) n3	0.31±0.02
Linoleic acid ($C_{18:2}$) n6	1.75±0.01
ΣPUFA	58.79
Saturated Fatty Acid	
Myristic acid (C_{14})	1.81±0.02
Pentadecanoic acid (C_{15})	1.97±0.01
Heptadecanoic acid (C_{17})	1.03±0.01
Stearic acid (C_{18})	9.22±0.01
Tricosanoic acid (C_{23})	2.31±0.02
Palmitic acid (C_{16})	14.85±0.09
Σ SFA	31.19
Σ n-3	51.24
Σ n-6	7.55
n3/n6	6.78
n6/n3	0.147

(All values are the means ±standard deviations of three replicates)

5.3.4. Physicochemical quality parameters of yellowfin tuna

The autolytic spoilage is responsible for the early loss of quality in seafood and is widely used as a quality index for fish. In marine fish, trimethyl amine oxide (TMAO) is broken down by autolytic enzymes but

mostly as a result of microbial action to form TMA, dimethyl amine (DMA), ammonia, formaldehyde and other volatile amines (Huss, 1994). In the present study TVB-N content of yellowfin tuna was 11.93 mg% (Table 5.5.) which was lower than the highest limit permitted by EC (EC directive 95/149/EC, 1995) for raw fish (35 mg %). Similar result was obtained for red drum (Li et al., 2013b). Among the chemical indices of spoilage, TMA is the one of the best spoilage indices produced by the bacterial breakdown of TMAO which is an osmolyte naturally found in marine fish. TMA is responsible for the fishy off flavor in spoiled seafood (Fu et al., 2007) and the rejection limit is usually from 5-10 mg% (El Marrakchi et al., 1990). TMA content of raw yellowfin tuna meat is presented in table 5.5. The results indicate that yellowfin tuna used in the study was of prime quality. A similar result was observed in Japanese sea bass (Cai et al., 2014).

One of the major reasons for the spoilage of fish is due to the oxidation of lipids. Oxidation of fat causes the formation of toxic compounds, changes in colour, taste, texture and nutritional value (Indergard et al., 2014). The auto oxidation of unsaturated lipids occurs by the action of oxygen. It leads to the formation of hydroperoxides, which further degrades to aldehydes and ketones causing strong rancid odour and flavor (Huss, 1994). The results of lipid oxidation parameters are shown in table 5.5. In the present study, primary oxidation of lipid was analyzed by measuring PV. The PV was not observed in the present study. A similar result was observed by Rodriguez-Turienzo et al. (2013). Lipid hydrolysis was determined by measuring the formation of FFA. In the present study the FFA observed for fresh yellowfin tuna meat was 2.24 mg% of oleic acid (Table 5.5). The formation of FFA itself does not lead to nutritional losses, but its measurement is important to measure the rancidity development. The pro-

oxidant effect of FFA on lipid mater has been explained by Aubourg (2001a). FFA have also been shown to undergo faster oxidation than intact lipids affecting the sensory quality of aquatic food products and provide greater accessibility to oxygen and other pro oxidant molecules. The secondary oxidation products of lipid were measured by TBA. The presence of TBA reactive substances (TBARS) is on account of the second stage of auto oxidation during which peroxides are oxidized to aldehydes and ketones (Li et al., 2013a). TBARS observed in the present study are presented in table 5.5. The value did not exceed 1-2 mg malonaldehyde kg^{-1}, which is normally regarded as the limit of acceptability (Connell, 1995).

Table 5.5 Physico-chemical quality parameters of raw yellowfin tuna.

TVBN (mg%)	12.25±0.05
TMA (mg%)	1.25±0.04
TBA (mg malonaldehyde.kg^{-1})	0.23±0.01
pH	6.53±0.8
FFA (mg% of oleic acid)	2.41±0.02
PV	Nil
Hardness 1 (kgf)	27.21±0.4
Hardness 2 (kgf)	24.31±0.21
Cohesiveness	0.371±0.03
Springiness (mm)	6.51±0.07
Springiness Index	0.54±0.01
Gumminess (kgf)	0.99±0
Chewiness (kgf.mm)	6.4±0.01
Fracture Force (kgf)	0.049±0
Adhesive Force (kgf)	0.039±0
Adhesiveness (kgf.mm)	0.036±0
Stiffness (kgf/mm)	0.541±0.01
L*	36.53±0.4
a*	6.46±0.2
b*	8.98±0.09
Overall sensory score	8.3±0.56

(All values are mean ±standard deviations of three replicates)

The pH of live fish muscle tissue is close to neutrality. pH of yellowfin tuna meat observed in the present study was 6.5 (Table 5.5). A similar result was also observed by Wei et al. (1990) for tuna meat. The colour of seafood is an important determinant of quality in seafood especially in tuna. The colour in seafood is influenced by muscle structure characteristics and pigment concentrations. In tuna, the colour changes from red/purple to cherry red to brownish red. This reflects the chemical oxidation state of myoglobin in the fish muscle (Anderson & Wu, 2005). In the CIE L*, a*, b* system, L* denotes lightness, a* denotes (+) red or (-) green, and b* denotes (+) yellow or (-) blue. The surface colour parameters of yellowfin tuna are shown in table 5.5. L* value of yellowfin tuna meat was 34.38 and a* and b* values were 5.67 and 8.15 respectively. From the result it was observed that there was no dark discolouration in the tuna meat used for study.

Food texture is defined as all the rheological and structural attributes of the product perceptible by means of mechanical, tactile and where appropriate, visual and auditory receptors (Chen & Opara, 2013). Texture analysis results of the present study are shown in table 5.5. Texture of fish muscle depends on numerous intrinsic biological factors like density of muscle fibers, fat and collagen content of fish muscle. Fish death triggers autolytic and microbial processes that make fish muscle softer and less elastic (Li et al., 2011). Hardness 1 of yellowfin tuna meat used for the study was 26.78 kgf. The springiness of sample was tested to simulate finger feel of raw sample. The sample showed a springiness value of 6.311mm. Chewiness represents the work done. The yellowfin tuna showed a chewiness value of 6.293 kgf mm. The gumminess value and fracture force was 0.99 kgf and 0.049 kgf respectively. Sensory attributes like appreance, colour, odour, taste

and texture were evaluated. The overall sensory score of fresh yellowfin tuna meat used for the study is shown in table 5.5. Yellowfin tuna meat had fresh seaweedy smell with firm texture and colour. The sensory score correlated well with other physico-chemical parameters.

5.4. Conclusion

In summary, yellowfin tuna is rich in crude protein with 23.18% and the percentage ratio of total essential amino acids to total amino acids was 48.2. Protein in yellowfin tuna muscle is well balanced with essential amino acids. The most predominant essential amino acids were lysine and leucine, while tryptophan was the essential amino acid with the lowest concentration. The n-3/n-6 ratio of yellowfin meat shows that it is a good source of PUFA. The most abundant mineral in yellowfin tuna meat was potassium followed by sodium. The average calcium and iron content in yellowfin tuna meat was 381.80 ppm and 101.86 ppm respectively. The other quality indices like TVBN, TMA-N, PV, FFA, pH, colour were well within acceptable limit, showing that the samples used in the present study were of good quality.

Chapter -6

SHELF LIFE EXTENSION OF YELLOWFIN TUNA (*Thunnus albacares* Bonnaterre, 1788) BY THE APPLICATION OF ACTIVE OXYGEN SCAVENGERS DURING CHILLED STORAGE

Content

Abstract

The application of oxygen scavengers on the shelf life extension of yellowfin tuna during chilled storage was compared with control air and vacuum packs. The commercial oxygen scavengers reduced the oxygen concentration inside the packs to below 0.01% within 24 hours and it maintained the reduced oxygen level till the end of storage period. Control air packed samples were sensorily acceptable up to 10 days and vacuum packed samples were acceptable till 12 days of chilled storage. The samples packed with commercial oxygen scavengers were acceptable till the end of storage period but the microbial count exceeded the acceptability limit on 18th day indicating a shelf life of 15 to 18 days. The oxygen scavenger significantly reduced the rate of fat oxidation, volatile base nitrogen formation and also

reduced the microbial growth during storage period. Thus, the application of oxygen scavengers were found to be effective in delaying the rate of spoilage compared to the control air and vacuum packed samples.

6.1. Introduction

Among tunas, yellowfin tuna *(Thunnus albacares)* are large pelagic fish that prevail in the tropics and subtropics (Al-Abdessalaam, 1995). Being one of the most highly perishable food product, spoilage will start immediately after catch. The degree of spoilage depends directly on the storage conditions (García-Soto et al., 2014). The loss of freshness and spoilage pattern of seafood varies with species. Fish spoilage normally follows four stages. Rigor mortis, resolution of rigor, autolysis and bacterial spoilage. The speed of these stages depends on species, physiological condition of fish, microbial contamination and temperature (Ocaño-Higuera et al., 2009). Temperature is one of the important factor which determines the spoilage rate of seafood. Hence it is important to reduce the temperature as quick as possible to preserve the initial freshness and quality of fish (Ocaño-Higuera et al., 2006). Storing fish at low temperature can prolong the shelf life of fish. According to Clucas & Ward (1996), for every 5^0C above 0^0C that the fish is stored, the storage life in ice is reduced by half. Icing is one of the most recommended method to keep the fish in cooled condition before freezing. The icing helps to maintain the uniform low temperature and helps to reduce the rate of autolysis. It also reduce the rate of bacterial action and provide a gentle washing effect during the melting of ice (Rand & Pivarnik 1992; Ghaly et al., 2010). The rate of spoilage during ice storage varies with species, which depends on the concentration of substrates, metabolites in the

tissue, microbial contamination and storage condition after harvest (Pacheco-Anguilar et al., 2000).

In many cases food deterioration is caused by the high levels of oxygen present in food packages which may facilitate microbial growth, off flavours and off odour development, colour changes, oxidation of food constituents thereby minimizing the shelf life of product (Kerry et al., 2006). Even though they are packed in modified atmosphere packs or vacuum packs, since these technologies do not always completely remove oxygen from package head space. Moreover oxygen that permeates through the packaging film cannot be removed by these technologies. Oxygen scavengers which absorbs residual oxygen after packing minimizes the quality changes of oxygen sensitive foods (Vermeiren et al., 1999). Hence the objective of the present study was to determine the effect of active oxygen scavengers on the shelf life extension of chill stored yellowfin tuna by monitoring the microbial, physicochemical and sensory changes as a function of packaging treatment.

6.2. Materials and methods

Yellowfin tuna was procured from Fort Cochin fish landing center, Kerala, India. The average weight of sample was 7.5±1.4kg with 71±3.8cm length and 20±1.4 cm width. Fish was washed in chilled potable water and brought to the laboratory in iced condition. Immediately after reaching the laboratory, fish were rinsed in chilled potable water and dressing was carried out manually. Yellowfin tuna chunks (100±10g) were then divided into three lots and packed separately. First batch was packed as control air packs, the second lot was vacuum packed and third batch was packed with commercial oxygen scavengers. The packaging material used for the study was EVOH

(Sealed air, India Pvt Ltd, Bangalore, India) with 0.14 mm thickness having O_2 permeability of 60.8 cc/m^2/24hr and CO_2 permeability of 96.18 cc/m^2/24hr (Table 4.1). Commercial O_2 scavenger sachets (Ageless® ZPT 200 EC O2 absorber) with O_2 absorption capacity of 200 ml supplied by Sealed air (India) Pvt Ltd, Bangalore, India were used for the study. One fish chunk was placed in each pouch (13 X 15cm) with one O_2 scavenger in active packs and sealed with sealing machine (Model No 405, Samarpan Fabarications Pvt Ltd, thane, India). Control air packs were packed without O_2 scavenger. Vacuum packs were sealed using a vacuum sealing machine (Vac Star S 210). Immediately after packing all packs were iced with flake ice in the ratio of 1:1 in an insulated box and were kept in chill room maintained at 0-2^{0}C. Ice melt water was removed every day and fresh ice was filled to maintain the temperature. Three pouches from all batches were drawn at three days intervals and subjected to head space gas composition, physicochemical, microbial and sensory quality evaluation.

Head space gas composition was measured with gas analyzer (PBI Dansensor, Checkmate 9900, Ronnedevej, Denmark) which functions based on a solid state O_2 ion conductive material (Zirconium oxide). Gas analysis was performed by drawing head space gas sample by piercing the syringe needle through a rubber septum provided by the company glued on the EVOH film. TVB-N and TMA were estimated by the micro diffusion method (Conway, 1962). TBA value of the sample was estimated spectrophotometrically (Tarladgis et al., 1960) and expressed as mg malonaldehyde kg^{-1} of sample. FFA was measured and expressed as mg % oleic acid (AOCS, 1989). PV was analysed and expressed as milli equivalent of O_2 kg^{-1} fat (AOCS, 1989).

Plate 6.1 Yellowfin tuna chunk packed in EVOH control air pack

Plate 6.2 Vacuum packed yellowfin tuna chunk in EVOH pouch

Plate 6.3 Yellowfin tuna chunk in EVOH pouch with commercial oxygen scavenger

pH was determined according to APHA (1998) using a digital pH meter (Cyberscan 510, Eutech instruments, Singapore) after homogenizing 10 g of the sample with the same amount of distilled water. The colour of the sample was measured with Hunter colorimeter (Hunter Lab colorimeter, MiniScan XE Plus Hunter Associates Lab inc., Reston, Virginia, USA). Water holding capacity was measured by placing 1 g sample on a pre-weighed filter paper and applying a pressure of 1Kg force for 60 seconds using a compression tester (Gaydon Compressor Tester, Model No 936, HE. Messer Ltd. London, UK). Drip loss was measured gravimetrically by taking the weight difference of the sample with and without exudate. The exudate was removed by draining and wiping the sample with filter paper.

Total plate count count was determined by spread plate method using plate count agar (PCA) as per AOAC (2000) method. Sensory analysis is one of the traditional method of judging the quality of fish. Sensory analysis of the samples was carried out by using a 9 point hedonic scale (annexure 1) as prescribed by Meilgaard et al. (1999).

All analysis were carried out in triplicate. Experimental data were analysed using the software Statistical Programme for Social Sciences (SPSS 20). For data analysis, mean, standard deviation and analysis of variance (ANOVA) were used. The least significant difference was calculated at the probability level $p<0.05$.

6.3. Results and discussion

6.3.1. Changes in head space gas composition

During storage, the head space gas composition of packs were analysed and the changes in head space gas composition of yellowfin tuna during chilled storage is shown in Fig. 6.1 and 6.2.

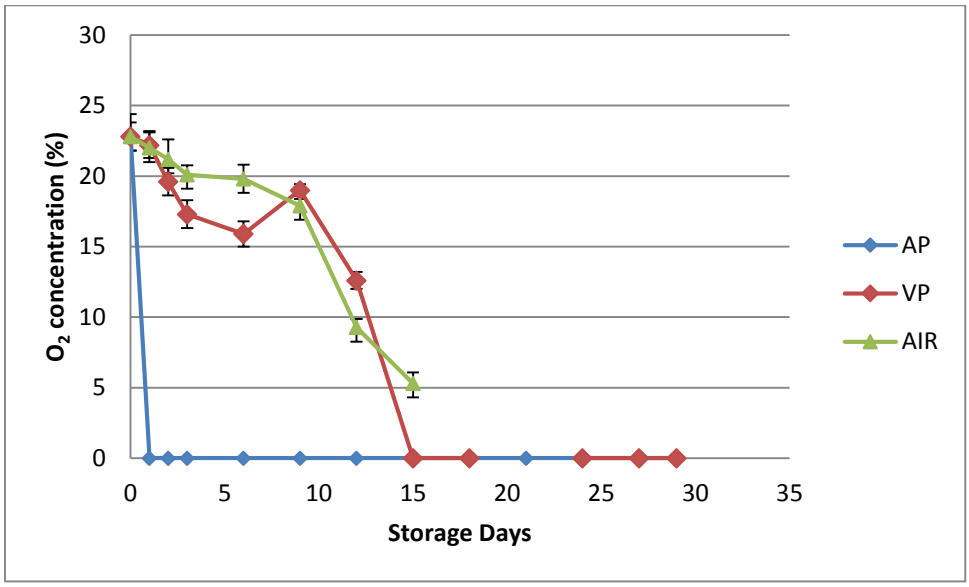


Fig. 6.1. Changes in head space gas composition (O_2)* of yellowfin tuna packs under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

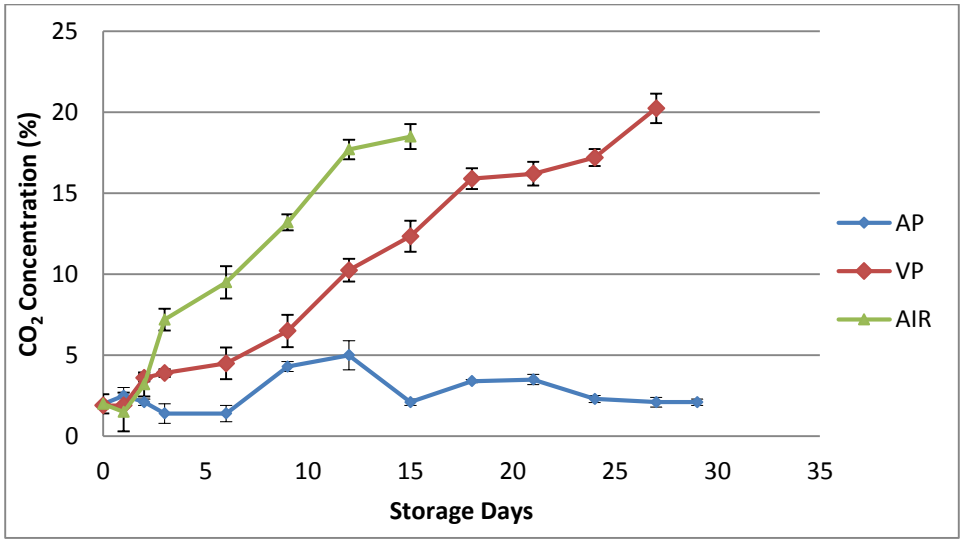


Fig. 6.2. Changes in head space gas composition (CO_2)* of yellowfin tuna packs under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

It was observed that the oxygen concentration of packs with oxygen scavenger was reduced to 0.01% within 24 hours of storage. Oxygen content in the packs was completely removed by second day and the oxygen free condition was maintained till the end of storage period in samples packed with commercial oxygen scavenger. The oxygen content of oxygen scavenger packs were significantly ($p<0.05$) different compared to control air packed samples. Vacuum and control packs showed a decreasing oxygen concentration during storage from the initial concentration of 22.8%. On 15th day, oxygen content was not observed in the vacuum packs. The decrease of oxygen concentration in control packs could be due to the microbial growth and by the oxidation processes (Torrieri et al., 2006; 2011). Similar results are reported for thread fin breams (Goncalves et al., 2004), cat fish (Mohan et al., 2008) and seer fish (Mohan et al., 2009a,b; 2010) cobia (Remya et al., 2017).

CO_2 concentration of samples packed in vacuum and control air packs showed an increasing trend during storage period. The increase in CO_2 concentration was significantly higher ($p<0.05$) for air packed control samples compared to oxygen scavenger packs. The CO_2 content remained almost constant in oxygen scavenger packs. The increase in carbon dioxide content in air packs may be due to the growth of aerobic spoilage microorganisms in the pack, which utilize O_2 and release CO_2. In oxygen scavenger packs, the low CO_2 content could be due to the effect of oxygen scavengers in preventing the growth of aerobic microorganisms. A similar result was also reported by Mohan et al. (2008; 2009a,b; 2010).

6.3.2. Changes in TVB-N

TVB-N is considered as a quality index for fish and its increase in fish is related to the activity of spoilage bacteria and endogenous enzymes

(Özogul et al., 2004; Ruíz-Capillas & Moral, 2005). According to Fraser & Sumar (1998), bacterial catabolism of aminoacids and nucleotide catabolites results in the accumulation of ammonia, methylamine, dimethylamine, and trimethylamine and other volatile bases. TVB-N produced during the storage period is shown in Fig. 6.3.

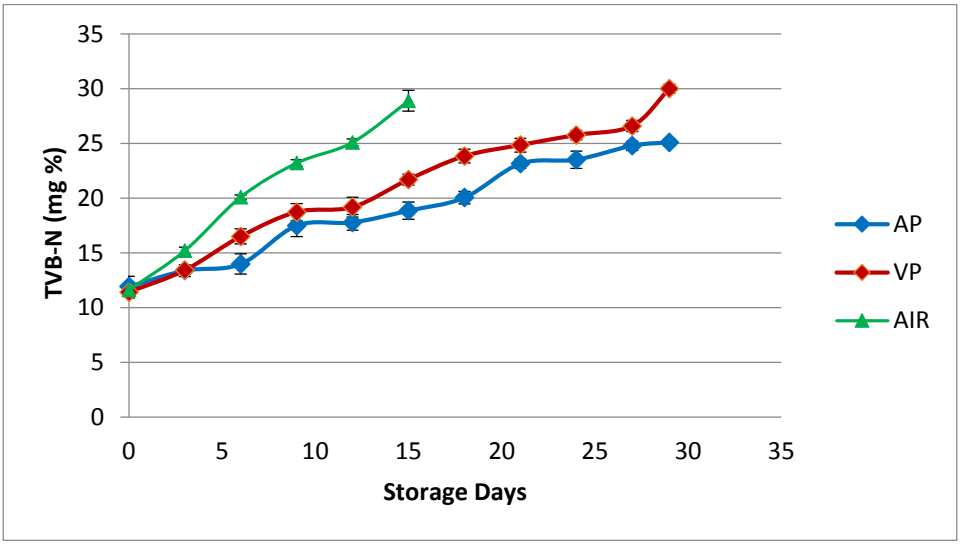


Fig. 6.3. Changes in TVB-N of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, $p<0.05$

An increasing trend was observed in all treatments with increase in storage time. Significant differences ($p<0.05$) were found between yellowfin tuna stored in air, vacuum and active packs during chilled storage. Yellowfin tuna stored in oxygen scavenger packs did not reach the highest level of acceptability during storage period. TVB-N values of marine fishes were reported to increase progressively as spoilage proceeds and a level of 30 mg N/100 g fish muscle is considered as the highest level of acceptability by Gökodlu et al. (1998). A similar increasing trend for TVB-N was also observed for gilthread sea bream (Grigorakis et al., 2003), sardine (Özogul et

al., 2004), European eel (Özogul et al. 2005) and white grouper (Özogul et al., 2008). The inhibition of TVB-N production in oregano oil treated vacuum packed trout samples during chilled storage was observed by Frangos et al. (2010). In the present study, the TVB-N values of active packed samples were well below the highest level of TVB-N acceptability limit when the microbial count exceeded the acceptable limit in samples. Hence it does not seem to be a major cause of spoilage of yellowfin tuna stored in active oxygen scavenger packs.

6.3.3. Changes in TMA

TMA is produced by the decomposition of trimethylamine oxide (TMAO) by bacterial spoilage and intrinsic enzymatic activity (Connel, 1990). The TMA formation in yellowfin tuna during storage showed an increasing trend in all packs (Fig. 6.4). In the present study the initial TMA was 1.3 mg%, which indicates that the yellowfin tuna used in the study was of good quality. The highest concentration of TMA was obtained for yellowfin tuna stored in air and the lowest was for samples stored in active packs. From the present study, the production of TMA was significantly ($p<0.05$) slower in active packed samples compared to the control air packed samples. TMA value did not reach the limit of acceptability (5-10 mg TMAN/100g, Sikorski et al., 1990b) in active packed samples on the day of sensory rejection.

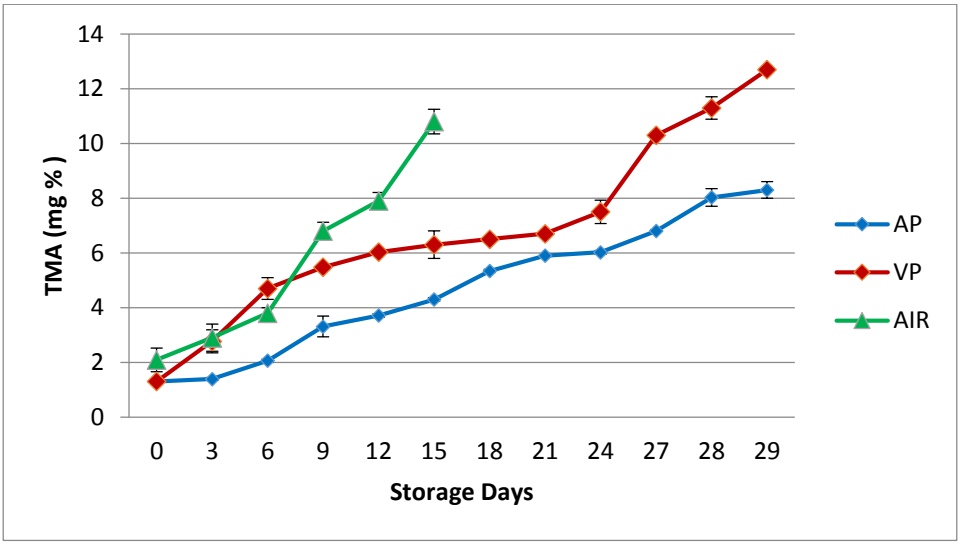


Fig.6.4. Changes in TMA of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

6.3.4. Changes in Lipid Oxidation

Lipids of fish muscle are prone to oxidation due to the higher content of unsaturated fatty acids in fish oil (Sohn et al., 2005) which is directly related to the production of off flavors and odors (Harris & Tall, 1994; Hsieh & Kinsella, 1989). There are two main reasons for lipid oxidation in food systems; they are enzymatic and non- enzymatic. The enzymatic oxidation occurs mainly at the site of oil-water interface and the second is induced by the presence of oxygen (Indergard et al., 2014). Oxidation of unsaturated fatty acids takes place in the presence of pro oxidants like heme proteins during storage. In fish the phospholipid membrane is believed to be the key substrate for lipid oxidation due to the presence of highly unsaturated fatty acids (Undeland et al., 1998). Lipid oxidation of yellowfin tuna during chilled storage was monitored by measuring lipid oxidation and hydrolysis products. Primary lipid oxidation

was monitored by changes in PV as shown in the Fig.6.5. The peroxide value indicates the amount of oxidized substances. Hydro peroxides are the primary products of auto oxidation and their decay leads to the formation of carbonyls compounds, hydrocarbons, furans and other compounds which contribute rancid taste and off flavours (Yanishlieva & Marinova, 2001). In the present study, an increase of PV was observed during the early days of storage. However the value started declining afterwards in all batches. The peroxide value of control sample were significantly higher ($p<0.05$) compared to the active oxygen scavenger packs, showing that active oxygen scavenger packs were effective in slowing down the rate of primary oxidation. According to de Abreu et al. (2010), PV tends to increase during the early stages of fat oxidation since the rate of formation of hydroperoxides are higher than the rate of decomposition. After reaching the maximum value, the value then decreases due to the lower availability of substrate and instability of peroxide molecule.

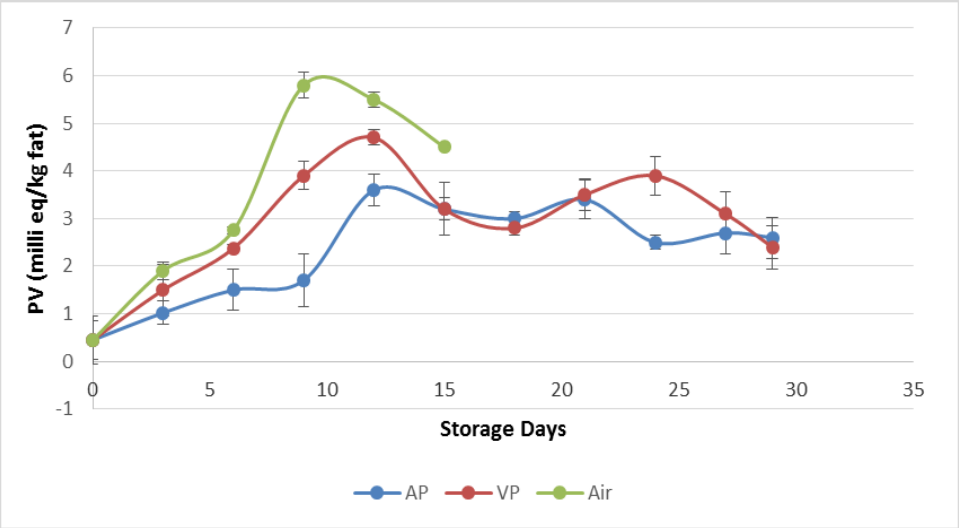


Fig.6.5. Changes in PV of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, $p<0.05$

The secondary lipid oxidation of lipid was measured by TBA (mg malondialdehyde/kg sample). TBA content in all samples showed an increasing trend during storage (Fig 6.6.). The concentration of TBA increased significantly ($p<0.05$) in control samples compared to vacuum and active oxygen scavenger packs. Any significant increase in secondary lipid oxidation compounds were not observed in samples packed in active packs. The TBA value of active oxygen scavenger packs never reached the highest level of acceptability during storage period. That may be due to the protective effect of oxygen scavenger towards secondary oxidation. According to Aubourg (1993), TBA values do not represent the actual lipid oxidation rate due to several interaction between amino acids, proteins, glucose and other fish constituents to form polymers that decrease the quality of fish (Fernandez et al., 1997; de Abreu et al., 2011b)

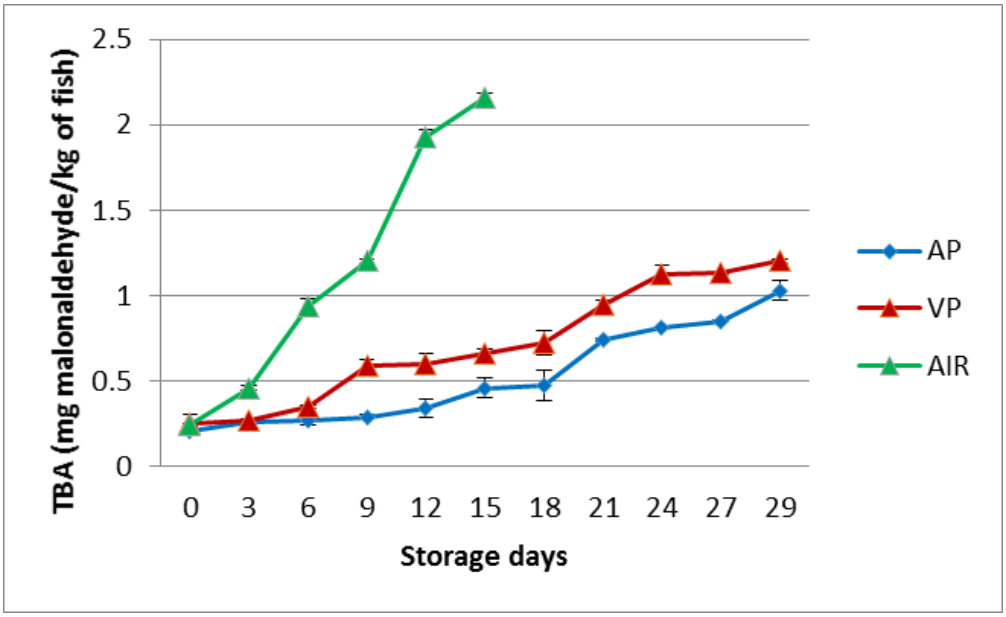


Fig.6.6. Changes in TBA of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, $p<0.05$

Lipid hydrolysis was determined by measuring FFA content. Progressive FFA formation was observed for all batches (Fig. 6.7.). The average initial FFA content of sample was 0.45 mg% oleic acid. A similar result was also reported for sardine (Aubourg et al., 1997), tuna (Gallardo et al., 1989), and horse mackerel (Losada et al., 2005). In the present study a significantly ($p<0.05$) lower FFA formation was detected in samples packed with active oxygen scavengers compared to vacuum and air packed samples. That may be due to the inhibitory effect of oxygen scavengers on lipid hydrolysis. FFA formation during chilled storage has been reported to be due to the result of endogenous enzyme activity and microbial action (Ashie et al., 1996; Whittle et al., 1990; García-Soto et al., 2013). The formation of FFA itself does not lead to nutritional loss, but is related to the texture deterioration by interacting with proteins (Mackie, 1993; Sikorski & Kolakowska, 1994) and accelerates lipid oxidation (Miyashita & Takagi, 1986; Yoshida et al., 1992).

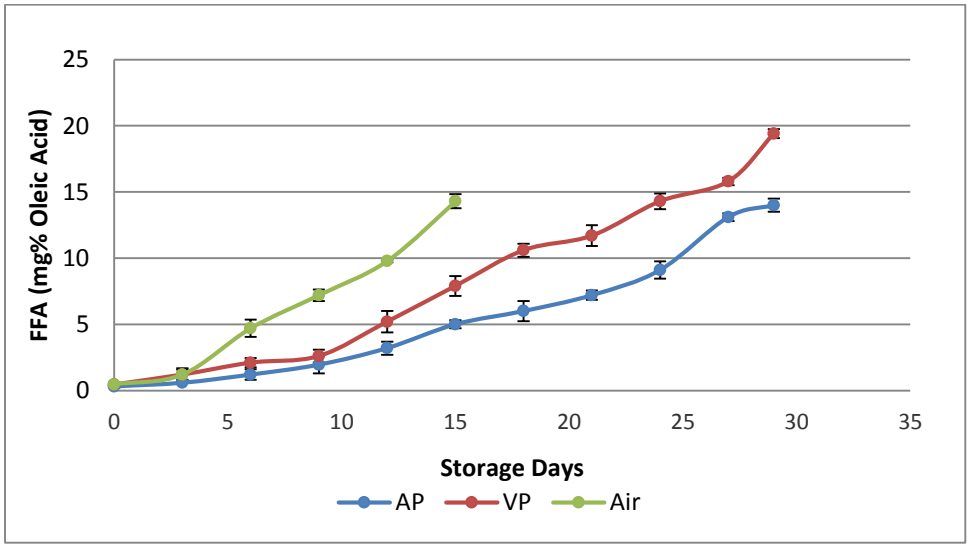


Fig.6.7. Changes in FFA of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, $p<0.05$

6.3.5. Changes in colour

Colour is one of the most important quality attributes of fish due to its relation with freshness and therefore has a direct effect on consumer's perceptions (Lawless & Heymann, 2010). The values of L*, a* and b* for samples packed in air, vacuum and active oxygen scavenger packs are given in Fig 6.8 a, b and c. The average initial L* value of yellowfin tuna during the present study was 36.65 and the value decreased with increasing storage days in all batches. A decreasing L* value was also observed for red snapper stored under modified atmosphere packs during chilled storage (Gerdes & Valdez, 1991) and for salted bonito under modified atmosphere packs (Caglak et al., 2012). The reduction of lightness is an indication of spoilage. Pigments like heme proteins are responsible for the colour in fish meat. Oxidation of pigments and loss of these pigments through drip loss is the cause of decreased lightness (Mohan, 2008).

Samples packed in air showed a significant ($p<0.05$) decrease in a* value during storage compared to the active packs. The reduction of a* value may be attributed due to the browning of yellowfin tuna meat during storage. According to Tajima & Shikama (1987), the browning is due to the oxidation of myoglobin, due to the contact with oxygen. The myoglobin reacts with oxygen to form bright red oxymyoglobin, which further oxidized to the brown ferric (Fe^{3+}) metmyoglobin. The objective of adding oxygen scavenger during the present study was to keep the tuna meat in oxidized oxymyoglobin state to maintain the attractive bright red colour of fresh tuna meat. So it is very important to reduce the oxygen concentration of packets as soon as possible to prevent the further oxidation of oxy myoglobin.

In the present study, the b* values showed an increasing trend during the study period. b* value increased from 6.85 to a maximum of 9.62 in control air packs. Among the samples packed in vacuum and active packs, there was no significant difference observed during the study period. Similar result was observed for seer fish steaks during chilled storage (Mohan, 2008).

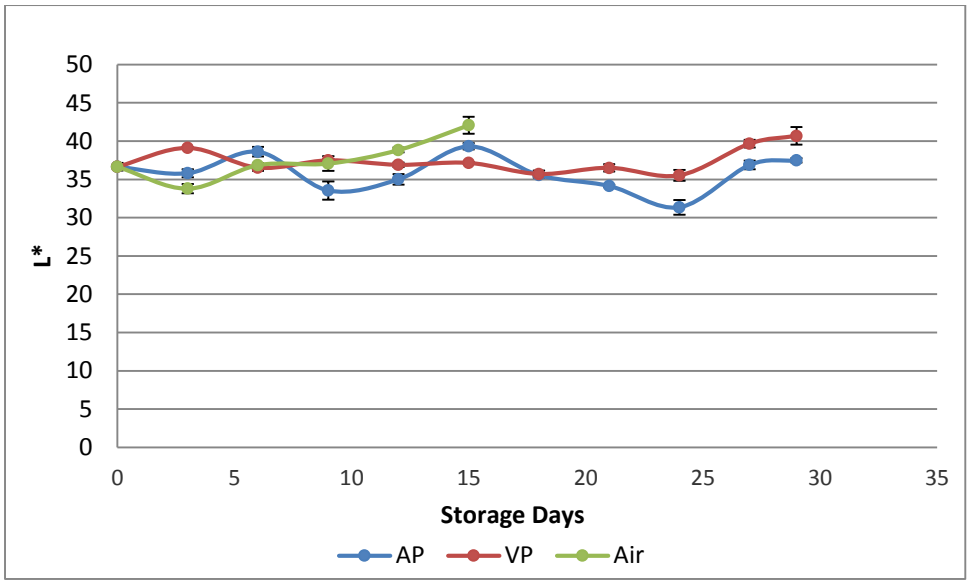


Fig.6.8a. Changes in L* value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

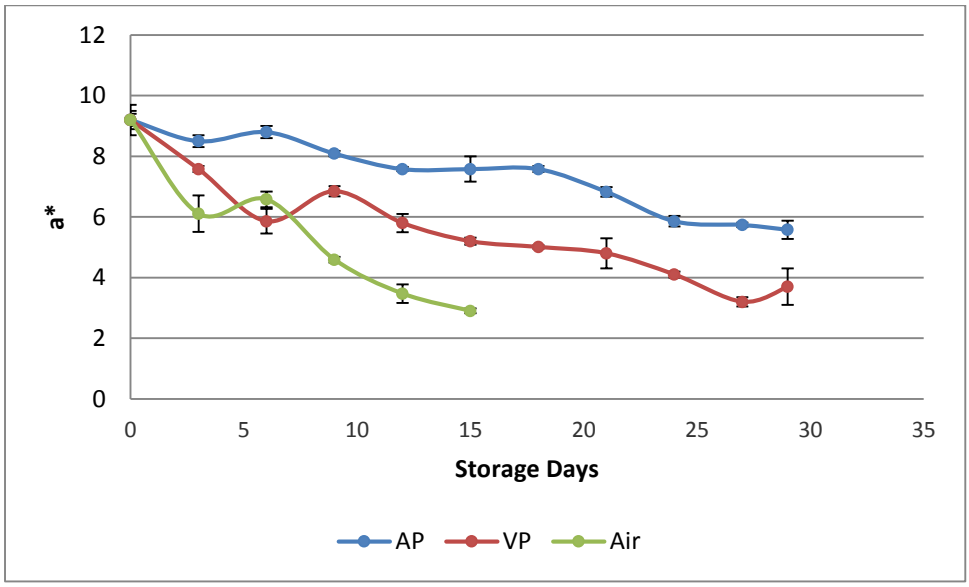


Fig.6.8b. Changes in a* value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

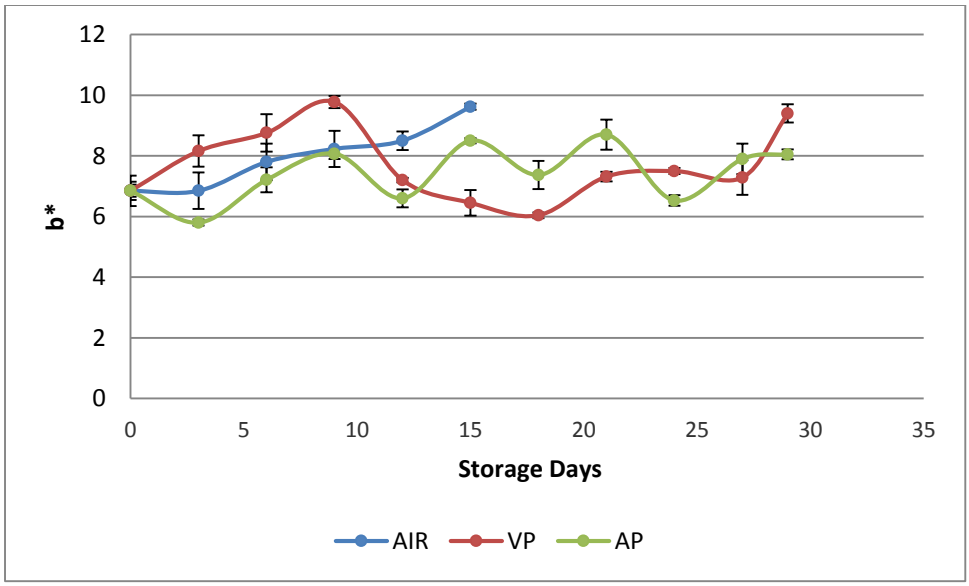


Fig.6.8c. Changes in b* value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

6.3.6. Changes in pH

There are Factors influencing the changes of pH during post motem, which include species, fish size, season, water composition, geographical location, and stress during catch (Li et al., 2013a). In the present study, initial pH of the sample was 6.52 (Fig. 6.9). pH values of all sample showed a decreasing trend during chilled storage. It may be due to the production of lactic acid by anaerobic glycolysis and liberation of inorganic phosphate by the degradation of ATP (Delbarre-Ladrat et al., 2006). Bacterial fermentation of carbohydrates resulting in the formation and accumulation of organic acids in fish meat leads to lowering of muscle pH (Khan et al., 2005). An increase of pH during the later stages of refrigerated storage of red drum fish (Li et al., 2013b) sea bream (Ayala et al., 2010), grass carp (Liu et al., 2013), sardine (Campos et al., 2005) was observed due to the formation of volatile basic compounds including ammonia, trimethyl amine, dimethyl amine etc. produced by either microbial or endogenous enzymes.

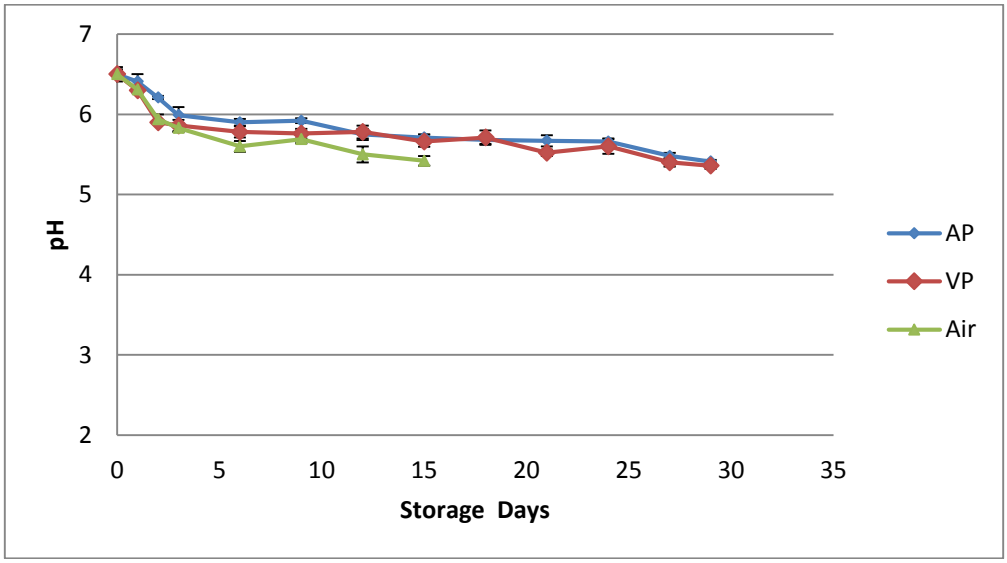


Fig.6.9. Changes in pH value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

6.3.7. Changes in water holding capacity

Water holding capacity of fish muscle is the ability of muscle to retain water even though external pressure is applied to it. Water holding capacity is closely related to textural properties. Change in water holding capacity of fish muscle during post mortem is due to structural changes in fish muscle (Duun & Rustad 2007; Shaviklo et al., 2010; Kaale et al., 2014). An absorbent method was used for measuring water holding capacity which consisted in applying 1 kg weight for 60 seconds on 1g sample that had been placed between two filter papers. The samples were weighed before and after the procedure. The changes in water holding capacity of yellowfin tuna during chilled storage is given in Fig. 6.10. The initial water holding capacity of yellowfin tuna was 14.79%, which was lower than the result reported for seer fish steak (Mohan 2008) and cat fish (Mohan et al., 2008). The water holding capacity of yellowfin tuna showed a decreasing trend during storage in all samples, however the rate of decrease was significantly higher ($p<0.05$) for control air packed samples. According to Sato et al. (1991), fish muscle usually become tougher when accompanied by a progressive loss of fluid and reduction of water holding capacity. Ocaño -Higuera et al. (2009) observed a reduction of water holding capacity of cazon fish muscle stored on ice due to the denaturation of myofibrillar protein during the period of storage. On the other hand, no significant difference in water holding capacity was observed for ray fish during ice storage (Ocaño -Higuera et al., 2011**).**

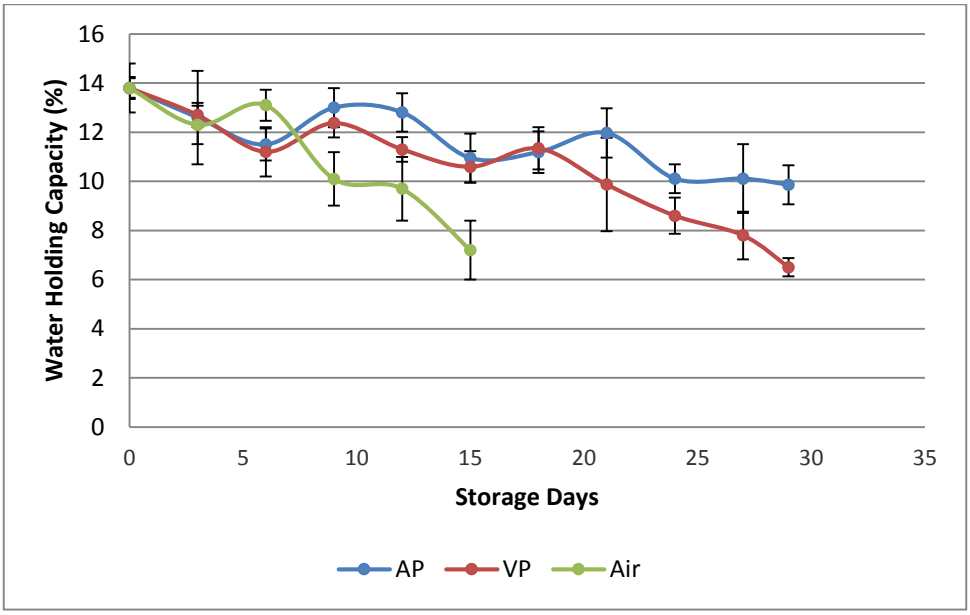


Fig.6.10. Changes in water holding capacity of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, $p<0.05$

6.3.8. Changes in drip loss

Drip is the water oozing from fish meat and is related to nutrient loss. Drip loss has been reported as an indicator for fish quality evaluation because the drip loss is associated with texture changes of fish muscle. Fig. 6.11 shows the changes of drip loss in yellowfin tuna during chilled storage at different packaging conditions. The drip loss increased significantly ($p<0.05$) in control air packed samples as a function of time. The initial drip loss was below 1% in all the three groups. A similar result was also observed for big head carp (Hong et al., 2013) and seer fish steaks (Mohan, 2008).

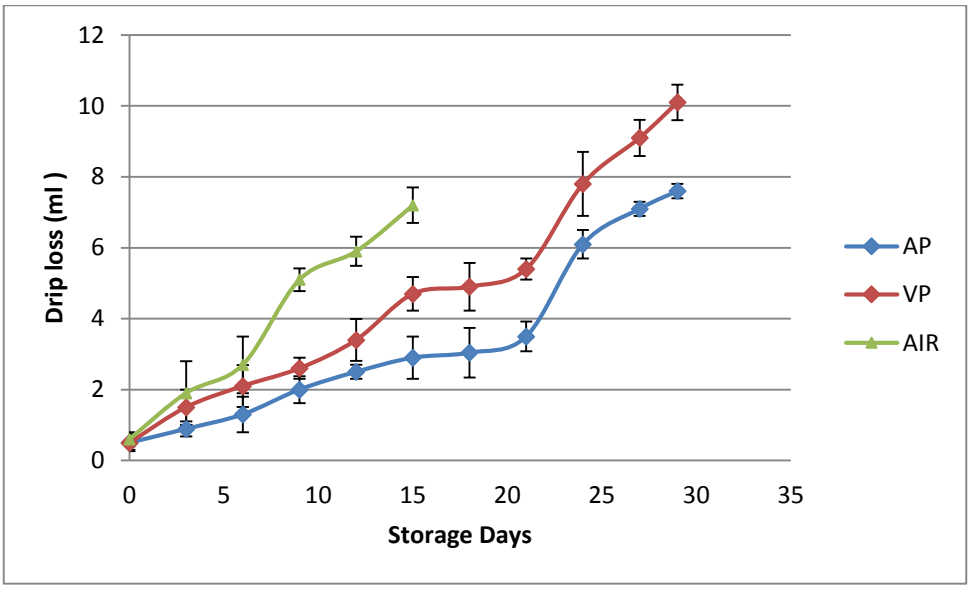


Fig.6.11. Changes in drip loss of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

6.3.9. Changes in total plate count

The growth and metabolism of microorganism trigger the spoilage of fish (Ghaly et al., 2010). The changes in the micro flora of yellowfin tuna during chilled storage is give in Fig. 6.12. There was a significant difference ($p<0.05\%$) observed between different treatments during storage period. The ICMSF (1998) has established a microbial acceptability of 7 log cfu /g for fresh water and marine species for human consumption. The initial total viable count value of yellowfin tuna muscle was 4.46 log cfu/g which indicated that the tuna used for the study was of good quality (ICMSF, 1998). The total plate count of yellowfin tuna samples increased gradually in all treatments during chilled storage as reported by Song et al. (2012) for sea bream, Özogul et al. (2008) for wild white grouper, Cai et al. (2014) for Japanese sea bass (*Lateolabrax japonicus*). The control

air packed samples showed a significantly higher ($p<0.05$) growth rate compared to oxygen scavenger packed samples. Air packed control samples exceeded the value of 7 log cfu/g on 9th day of storage indicating the shelf life between 6 to 9 days. This value is in agreement with those obtained for whole aqua cultured rainbow trout (*Onchorynchus mykiss*) during ice storage (Chytiri et al., 2004), European sea bass (*Dicentrarchus labrax*) stored in ice (Kyrana & Lugovois, 2002) and for yellow coaker (*Pseudosciaena crocea*) stored at 4^{0}C (Li et al., 2013a). The active oxygen scavenger packed samples exceeded the acceptability limit on 18th day of chilled storage indicating a shelf life of 15 to 18 days. Vacuum packed samples exceeded the microbial limit on 12th day of storage, which indicates a shelf life of 9 to 12 days.

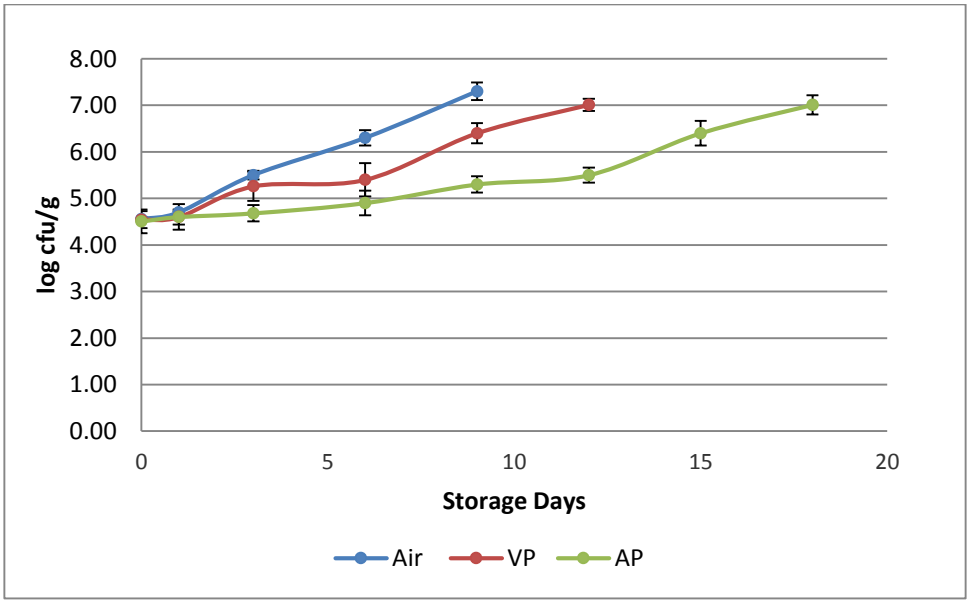


Fig.6.12. Changes in total plate count of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

6.3.10. Sensory analysis

The result of sensory evaluation of yellowfin tuna during storage is presented in Fig. 6.13. The overall acceptability score decreased in all samples with storage period and active packed samples showed a significantly ($p<0.05$) higher score than the control air and vacuum packed samples during storage period. It can be seen that samples packed in active packs retained good quality up to 20 days and were sensorily acceptable till the last day of storage. However the vacuum packed and control air packs were acceptable up to 12 and 10 days respectively. According to Özogul et al. (2004) the shelf life of sardine stored in vacuum packs was 9 days and 3 days for control air packs. Clingman & Hooper (1986) found a shelf life extension of 7 days for fresh fish products stored in vacuum packs. Lougovois et al. (2003) reported 16 days shelf life for iced ungutted sea bream. Özogul et al. (2006c) observed 12-15 days of acceptable shelf life for iced gutted trout and Tzikas et al. (2007) found 10 days shelf life for iced whole Mediterranean horse mackerel and 7 days for iced blue jacked mackerel. In the present study, results of sensory evaluation correlated well with increase in total plate count and TVB-N formation in the case of control air and vacuum packed samples. However, the active oxygen scavenger packs were sensorily acceptable till the end of storage period. The microbial count of oxygen scavenger packed samples exceeded the microbial acceptable limit on 18^{th} day. Even though the active packed samples were sensorily acceptable, the microbial count limited the shelf life of yellowfin tuna samples to 18 days.

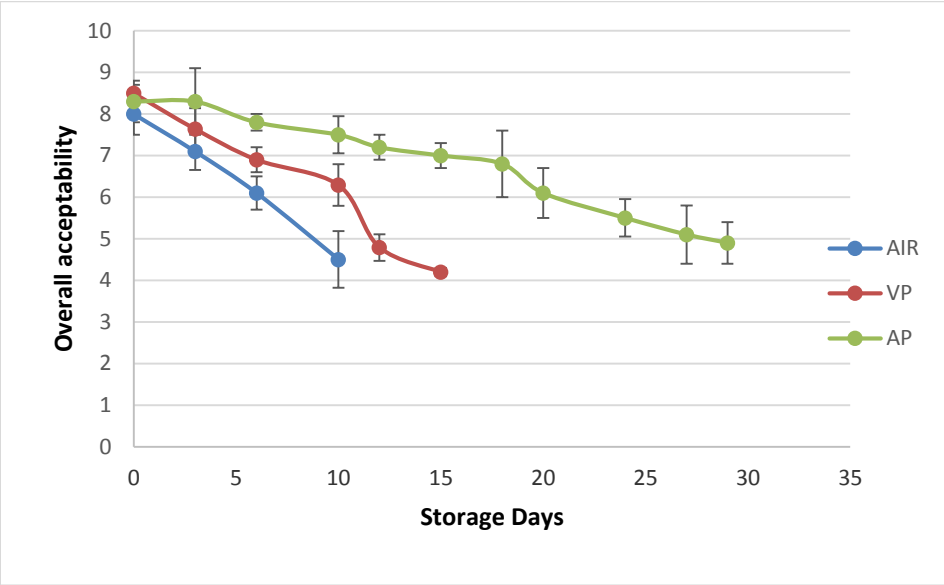


Fig.6.13. Changes in sensory score of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, $p<0.05$

6.4. Conclusion

The study indicated that yellowfin tuna packed with O_2 scavenger helped in maintaining the sensory, microbial and physico-chemical quality compared to vacuum and control air packs. Significantly ($p<0.05$) lower values were observed for volatile bases and lipid oxidation products for tuna packed with commercial O_2 scavenger. In vacuum and control air packs, both sensory and microbial quality were the major attributes deciding the shelf life, wheras in active packed samples, shelf life was limited by microbial quality. The results demonstrated the efficiency of oxygen scavengers in slowing down the rate of spoilage.

Chapter - 7

EFFECT OF OXYGEN SCAVENGERS ON THE MICROBIAL QUALITY OF YELLOWFIN TUNA (*Thunnus albacares* Bonnaterre, 1788) DURING CHILLED STORAGE

Contents

Abstract

The effect of oxygen scavengers on the microbial quality of yellowfin tuna was determined during chilled storage. The results were compared with control air and vacuum packs. The oxygen scavenger reduced the oxygen content inside the packs to less than 0.01% within 24 hours of storage. The microbial count crossed the upper acceptability limit on the 18^{th} day 12^{th} day and 9^{th} day of storage in the case of samples packed with oxygen absorber, vacuum pack and air pack respectively. H_2S producing bacteria were dominant in control air packs in the present study. In samples packed with oxygen absorber, Lactic acid bacteria were dominant followed by *B thermosphacta* during storage and samples were sensorily acceptable until

the last day of storage. samples packed with vacuum and control air were sensorily acceptable up to 12 and 9 days respectively

7.1 Introduction

Fresh foods especially seafood are highly perishable due to their biological composition. The quality of sea food decline rapidly during postmortem due to various microbial and biochemical action (Pigott & Tucker, 1990; Rey et al., 2012). Bacteria responsible for fish spoilage in marine fish varies according to the harvest environment, post process handling, degree of cross contamination and the preservation methods adopted post harvest. Controlling microbial activities is important for the shelf life extension during processing, distribution and storage (Kaale et al., 2011). According to IFST (1993), shelf life is defined as the period under defined conditions of storage for which a food product remains safe and fit for use. Shelf life of seafoods can be prolonged by modifying the gaseous atmosphere around it (Clingman & Hooper, 1986; Kristinsson et al., 2006). This can be done by removing oxygen with vacuum packing or by modifying the atmosphere inside by modified atmospheric packing to retard the aerobic spoilage microorganisms and oxidative chemical reactions (Parkin & Brown, 1982; Smith et al., 1990; Kristinsson et al., 2008). However, these technologies do not completely remove oxygen from the package. Oxygen permeates through the packaging material during storage and this cannot be removed by these techniques (Vermeiren et al., 1999). Active packaging is a novel packaging technology having been successfully used to extent the shelf life of seafood. It is defined as a type of packaging that changes the condition of the packaging to extend shelf life or to improve safety or sensory properties while maintaining the quality of the packaged food (Ahvenainen, 2003). Oxygen absorbers are the most widely used active packaging systems.

Oxygen scavengers are used to absorb residual oxygen after packing to control the growth of aerobic spoilage microorganisms and other oxidative deteriorations.

Yellowfin tuna is commercially important fish species and being a highly perishable commodity, it has to be processed and packed immediately after harvest. Microbial spoilage causes the production of off flavours and off odours during storage. Removal of oxygen inside the pack with proper temperature control can inhibit the growth of aerobic spoilage microorganisms (Caglak et al., 2012). Hence the objective of the present work was to determine the effect of oxygen scavengers on the microbial quality of yellow fintuna during chilled storage. The results were compared with vacuum and control air packs.

7.2 Materials and methods

Yellow fin tuna with average weight of 7.5±1 kg, 71±3.5 cm length and 20±1.5 cm width procured from Fort Cochin fish landing center, Kerala, India was served as research material throughout the study. Samples were washed in chilled potable water and brought to the laboratory in iced condition. Immediately after reaching the laboratory, fish were washed in chilled potable water and dressed manually. Yellow fin tuna chunks (100±10g) obtained were then divided into three lots and packed separately. The packaging material used for the study was EVOH (Sealed air, India PVT Ltd, Bangalore, India) with 0.14 mm thickness having O_2 permeability of 60.8 cc/m^2/24hr and CO_2 permeability of 96.18 cc/m^2/24hr. One fish chunk was placed in each pouch (13 X 15cm). First lot was packed as control air packs, the second lot was vacuum packed and the third lot was packed with oxygen scavengers. Control air packs were sealed with sealing machine

(Model No 405, Samarpan Fabarications Pvt Ltd, thane, India). Vacuum packs were sealed using a vacuum sealing machine (Vac Star S 210). Commercial O_2 scavenger sachets (Ageless® ZPT 200 EC O_2 absorber) with O_2 absorption capacity of 200 ml supplied by Sealed air (India) Pvt Ltd, Bangalore, India were used for the study. Each pouch in the third lot was packed with O_2 scavenger sachet and sealed with sealing machine (Model No 405, Samarpan Fabarications Pvt Ltd, thane, India). Immediately after packing all packs were stored under ice (flake ice) in the ratio of 1:1 (fish:ice) in an insulated box and were kept in chill room maintained at 0-2^{0}C. Ice melt water was removed every day and fresh ice was filled to maintain the quality of icing. Three pouches each from three batches were drawn at three days intervals and subjected to analysis.

Gas analysis was performed by drawing head space gas sample by piercing the syringe needle through a rubber septum provided by the company glued on the EVOH film and analyzing with gas analyzer (PBI Dansensor, Checkmate 9900, Ronnedevej, Denmark) which functions based on a solid state O_2 ion conductive material (Zirconium oxide).

Microbiological analysis

Ten gram of fish sample was aseptically weighed and homogenized with 90 ml of normal saline (0.85%) for one minute in a stomacher at 230 rpm (Seward Stomacher 400 Circular, London, UK). The homogenized sample was serially diluted using sterile 9ml saline for bacteriological analysis.

Total mesophilic count was determined by the spread plate method using Plate count agar (PCA) (Difco) as per Townley & Lanier (1981). The average counts were calculated and expressed as colony forming

units per gram (log cfu/g) of the sample. Enterobacteriaceae were enumerated on Violet Red Bile Glucose Agar (VRBGA, Oxoid, CM 485) as per Koutsoumanis & Nychas (1999). The average counts were calculated and expressed as colony forming units of Enterobacteriaceae per gram (log cfu/g) of the sample. Hydrogen sulphide producing bacteria were enumerated on Peptone Iron Agar (PIA) (code 289100, BBL Difco) with added 1% salt (Gram et al., 1987). Black colonies formed by the production of H_2S were enumerated (Gennari & Campanini, 1991) and the counts were expressed as number of colony forming units of H_2S producing bacteria per gram of the sample (log cfu/g). Lactic acid bacteria were enumerated on MRS agar (de Man, Rogosa and Sharpe, Himedia (code M 641) supplemented with potassium sorbate as per Mossel (1987). Pure white colonies (2-3mm dia) were counted as *Lactobacillus spp*. The average count was calculated and expressed as colony forming units per gram (log cfu/g). For the enumeration of *Brochothrix thermosphacta*, Streptomycin Thallus Acetate Acetidione Agar (STAA) was used (Mead & adams, 1977). Samples (0.5ml) of appropriate serial dilution were added in duplicate to pre dried sterile STAA plates and spread evenly using sterile bend glass rod. The plates were then incubated at 20°C for 5 days. To the incubated plates cytochrome oxidase reagent was added and the white colonies were counted as *B. thermosphacta*. Counts were expressed as number of colony forming units per gram of fish (log cfu/g).

Faecal streptococci were enumerated on Kenner Faecal Agar (KF, BBL, Difco) USFDA, (1995). All red to pink colonies were counted as *Faecal Streptococci*. The average count of duplicate samples was calculated and expressed as colony forming unit of *F. streptococci* per gram (log cfu/g) of the sample. *Faecal streptococci* was determined only for fresh fish and for

fish at the time of sensory rejection. *E coli* was enumerated on Tergitol 7 (T7) agar (Surendran et al., 2009). Sampling was carried out for fresh fish and for fish at the time of sensory rejection. Colonies with lime yellow, occasionally with rust brown center and yellow zone around were isolated and confirmed as *E coli* on EMB agar plates and by IMViC test. *Staphylococcus aureus* was enumerated on Baired Parker Agar (BP, Difco) (AOAC, 2000). Sampling was carried out for fresh fish and for fish at the time of sensory rejection. The black, convex colonies with clear margin zone were counted as *Staphylococcus aureus.* Typical colonies were isolated and confirmed by coagulase test. Counts were expressed as number of colony forming units of staphylococcus per gram (log cfu/g) of the sample. *Clostridium botulinum* toxin was detected by mouse bioassay as per the procedure described in US Food and Drug Administration Bacteriological Analytical Manual (USFDA, 2001). Sensory analysis of the samples were carried out using a 9 point hedonic scale (annexure 1) as prescribed by Meilgaard et al. (1999).

Three pouches from each batch were sampled randomly for the analysis. Statistical analysis was based on triplicate analysis for each sample at each specific storage time. Experimental data were analysed using the software IBM SPSS 20. Analysis of variance was performed and results are expressed as mean ±standard deviation. The level of significance was set at 5%.

7.3 Results and discussion

7.3.1 Changes in head space gas composition

During storage, the headspace gas composition of packs were analysed and the changes in headspace gas composition of yellow fin tuna during chilled storage is shown in Fig. 7.1 and 7.2. It was observed that

oxygen concentration in packs with oxygen scavenger was reduced to 0.01% within 24 hours of storage. Oxygen content inside the packs were completely removed within two days and oxygen free condition was maintained until the end of storage period. The oxygen concentration of vacuum and control packs also showed a decreasing trend during storage from an initial concentration of 22.1%. The decrease in O_2 concentration was significantly different ($p<0.05$) in samples packed with commercial oxygen scavenger compared to control air and vacuum packed samples. The decrease of oxygen concentration in control packs could be due to the microbial growth and by the oxidation processes (Torrieri et al., 2006; Torrieri et al., 2011). Similar results were reported for thread fin breams (Goncalves et al., 2004), cat fish (Mohan et al., 2008) and seer fish (Mohan et al., 2009, 2010). CO_2 concentration of samples stored under vacuum and air showed an increasing trend during storage period while the CO_2 content remained almost constant in active oxygen scavenger packs. The CO_2 level increased from an initial content of 2% to 20% in 15 days in air packs. The increase of CO_2 inside the packs was significantly faster ($p<0.05$) in air packs compared to commercial oxygen scavenger packs. The increase in carbon dioxide content could be due to the growth of aerobic spoilage microorganisms in the pack, which utilize O_2 and release CO_2. In oxygen scavenger packs, the low CO_2 content observed may be due to the inhibition of growth of aerobic microorganisms in the oxygen free conditions. A similar result was also reported by Mohan et al. (2009; 2010).

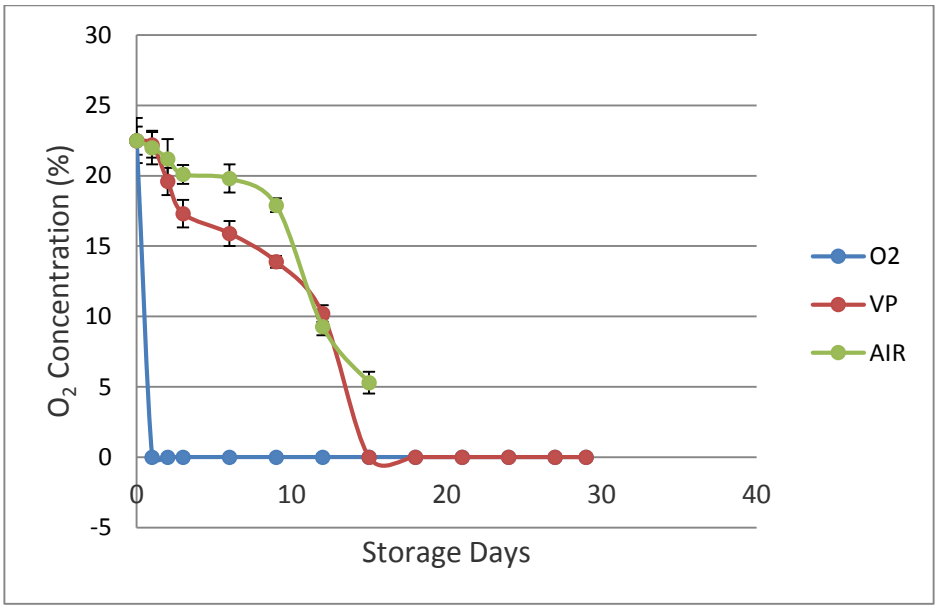


Fig.7.1. Changes in head space gas composition (O_2)* of yellowfin tuna packs under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3

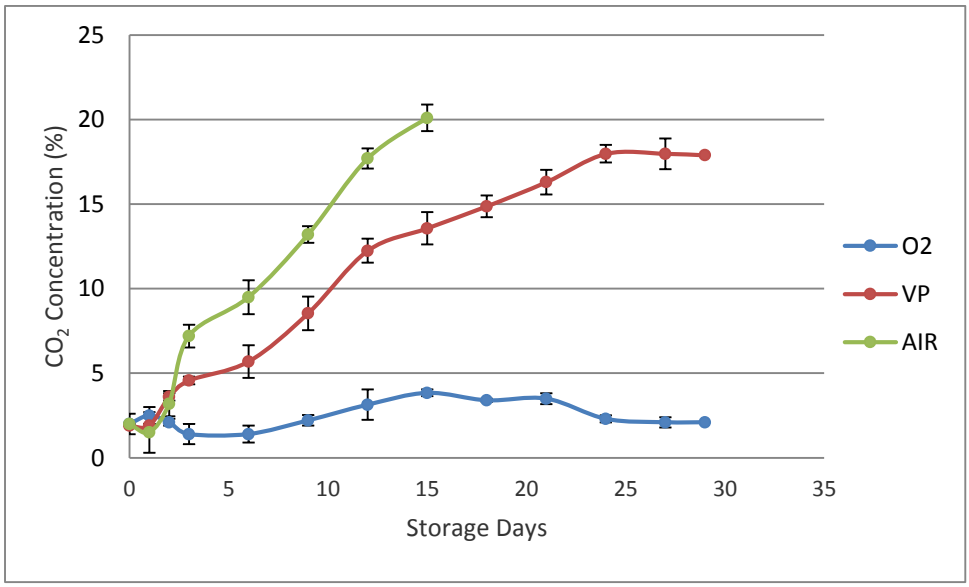


Fig. 7.2. Changes in head space gas composition (CO_2)* of yellow fin tuna packs under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, O_2 = samples with commercial oxygen scavengers) *Mean ± sd, n = 3

7.3.2 Changes in Mesophilic bacterial count

The initial mesophilic count of yellowfin tuna was 4.56 log $cfug^{-1}$. The changes in the total mesophilic count of yellowfin tuna during chilled storage is shown in Fig. 7.3. A significant increase ($p<0.05$) in mesophilic count over the whole storage period was observed in the present study. In control air packs, a steady increase in bacterial count was observed without any lag phase. However the microbial count of samples packed with oxygen absorber was significantly lower ($P<0.05$) than air packed control samples. A one-log increase in total mesophilic count was observed after 12^{th} day of storage and the counts increased gradually in active oxygen scavenger packs. Lag phase of 3 days were observed for samples packed with commercial oxygen scavengers. The lag phase observed in the oxygen scavenger packs may be due to the altered environment inside the pouches on the growth of microbes. Control and vacuum packed samples reached the upper acceptability limit for fresh marine species (7 log $cfug^{-1}$) considered by ICMSF (1986) on 9^{th} and 12^{th} day of storage respectively. The microbial count crossed the upper acceptability limit on the 18^{th} day of storage in the case of samples packed with oxygen absorber.

It is widely accepted that the initial microbial load of seafood is closely related to the water conditions and temperature and it ranges between 10^2-$10^4 cfug^{-1}$ (Savvaidis et al., 2002; Li et al., 2013a). Similar results were also reported for seer fish steaks (Mohan et al., 2010; Yesudhason et al., 2009), pearl spot (Manju et al., 2007a) and sea bass (Papadopoulos et al., 2003). Fernandez et al. (2009) observed twentytwo days of shelf life for salmon packed in modified atmosphere packs with less oxygen content. The results obtained in the present study are comparable with the results reported for seer fish packed with oxygen scavengers (Mohan et al., 2010). The study

indicates that the oxygen absorber is effective in inhibiting microbial growth significantly (P<0.05) by extending the lag phase and the generation time in yellowfin tuna chunks during chilled storage compared to the control air packs. The growth and metabolism of microorganisms induces the spoilage of seafood and almost 30% of landed fish are lost as a result of microbial activity (Ghaly et al., 2010; Li et al., 2013a).

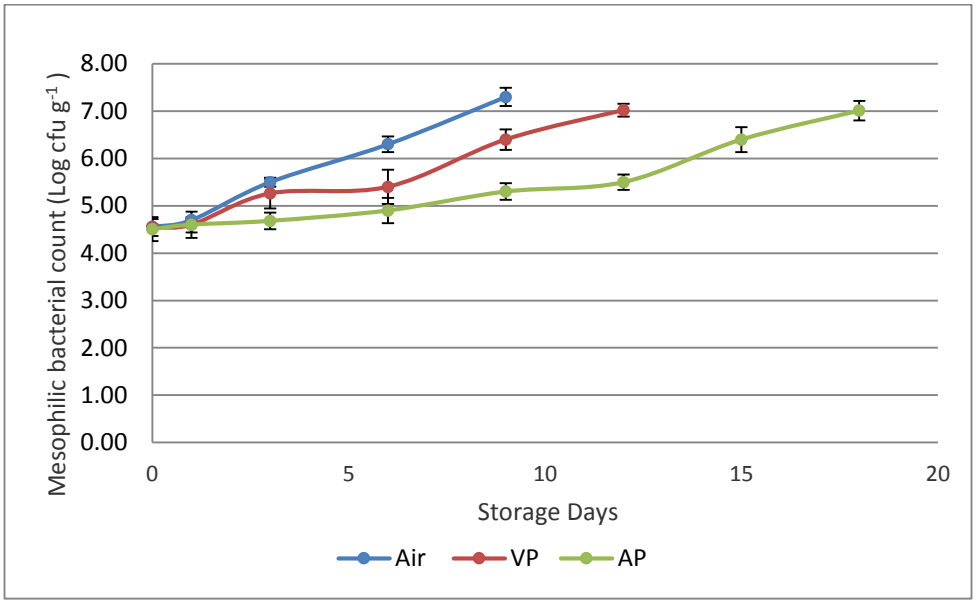


Fig 7.3. Changes in the total mesophilic count of yellowfin tuna chunks packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

7.3.3 Changes in H_2S producing bacteria

Changes in H_2S producing bacteria in yellowfin tuna chunks during chilled storage is shown in Fig.7.4. The initial count of H_2S producing bacteria was 3.95 log cfu g^{-1}. A lower initial count of 2.3 log cfu g^{-1} was reported by Pantazi et al. (2008) in sword fish and higher initial count of 4.3 log cfu g^{-1} was reported by Yesudhason (2007) for seer fish. A lag phase of six days was observed for samples packed with oxygen absorbers, whereas

no lag phase was observed for samples packed with vacuum and control air packs. This could be attributed to the inhibitory effect of oxygen scavengers on this group of bacteria. H_2S producing bacteria were found to be one of the dominant microflora in control air packs in the present study ($p<0.05$). The bacterial count increased steadily in control samples and reached 7.1logcfug^{-1} in at the time of sensory rejection. On the contrary, lower H_2S bacterial count was reported for European sea bass during the time of sensory rejection (Kyrana & Lougovois, 2002). In oxygen scavenger packed samples, the count reached 6.27 log cfug^{-1}on 23rd day. The reduction of bacterial growth and the extension of lag phase may be due to the effect of oxygen scavengers. H_2S producing bacteria are considered as one of the specific spoilage bacteria in fish from temperate and tropical waters (Lima & CAM, 1981; Gram & Huss, 1996).

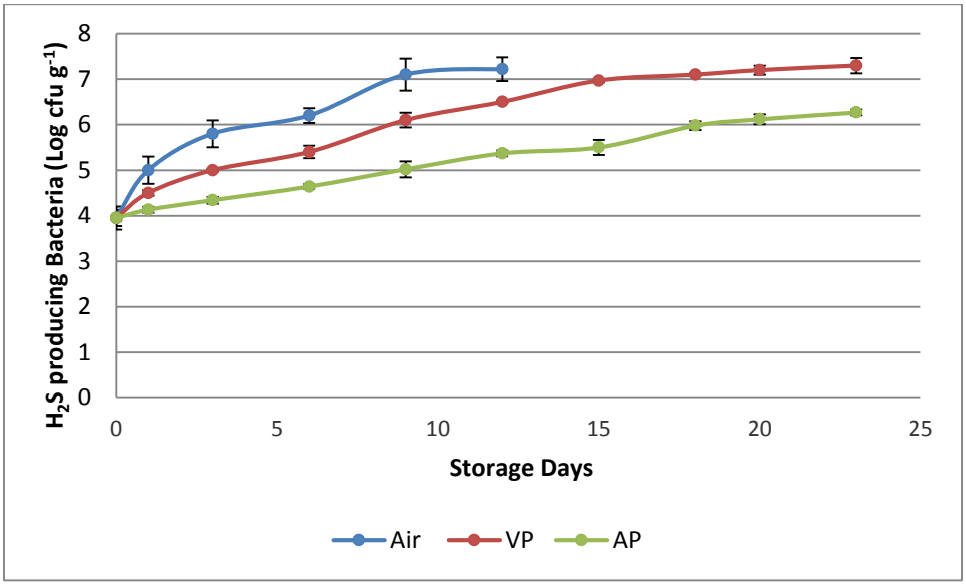


Fig 7.4. Changes in H_2S producing bacterial count of yellowfin tuna chunks packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

7.3.4 Changes in Enterobacteriaceae

Enterobacteriaceae play an important role in seafood spoilage because of their ability to metabolize amino acids to malodorous volatile compounds (Baylis, 2006; Remenant et al.,2014). The initial enterobacteriaceae count of yellowfin tuna in the present study was 2 log cfug^{-1} and it reached 3.5, 3.2 and 2.5 log cfu g^{-1} on the first day of storage for control air, vacuum and active oxygen scavenger packs respectively (Fig. 7.5). In control air packed samples, the count increased and reached 4.03 log cfug^{-1} on 9th day of storage. A similar result was observed for gilthead sea bream (*S aurata)* (Tejada & Huidobro, 2002), rainbow trout (*O mykiss*) (Chytiri et al., 2004), yellow grouper (*E awoara*) fillets (Li et al., 2011), during storage. A much higher enterobacteriaceae count was observed for sole and hake fillets during chilled storage (Fernandez-Saiz et al., 2013). Growth of enterobacteriaceae was retarded during subsequent storage in oxygen scavenger packs. This could be due to the protective effect of low oxygen against the growth of enterobacteriaceae. Similar result was also reported for seabass (Papadopoulos et al., 2003), Mediterranean boque (Koutsoumanis & Nychas, 1999) and for seer fish (Mohan et al., 2010). According to Papadopoulos et al. (2003), even though the enterobacteriaceae can grow at lower temperatures, their abundance decrease during ice storage due to their lower growth rate than that of other gram negative psychrotrophic spoilers and their inability to compete with the other gram negative psychrotrophic spoilers.

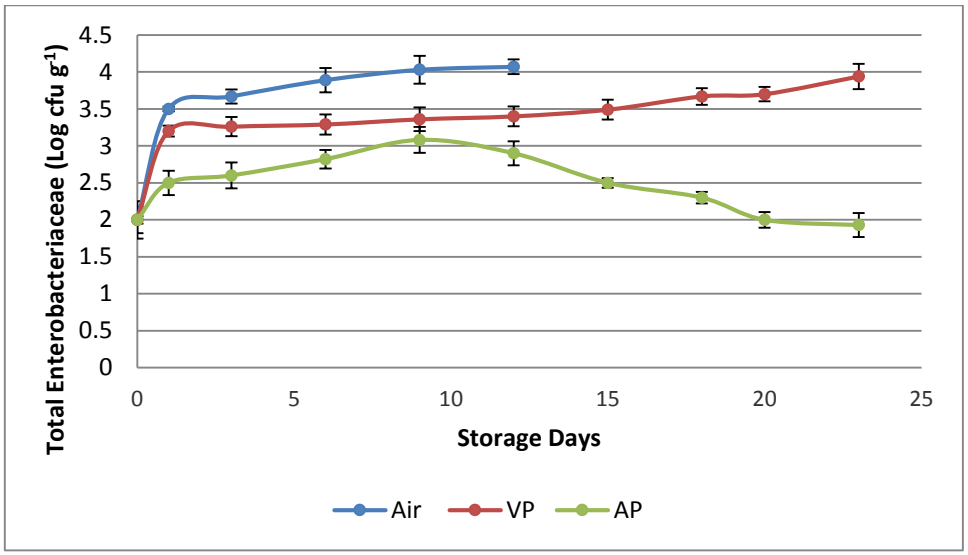


Fig 7.5 Changes in the total enterobacteriaceae count of yellowfin tuna chunks packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

7.3.5 Changes in *Faecal streptococci*

The initial *Faecal streptococci* count in Yellowfin tuna was 3.54 log cfu g^{-1}, slightly higher to the results reported for pearl spot (Manju et al., 2007a) but similar result was reported by Yesudason (2007) for seer fish and Manju (2005) for black pomfret. At the time of sensory rejection, faecal streptococci showed a significantly higher ($p<0.05$) value for control air packs reaching a maximum value of 5.7 log cfu g^{-1}, 5.41 log cfu g^{-1} for vacuum and 5 log cfu g^{-1} for active packed yellowfin tuna samples. A similar result was also reported for vacuum packed sodium acetate treated black pomfret and pear spot (Manju, 2005), modified atmosphere packed seer fish (Yesudhason, 2007) and oxygen scavenger packed seer fish steaks (Mohan et al., 2010). The results of the present study indicated that storage of yellow fin

tuna under active packs with reduced oxygen concentration does not increase the risk from streptococci above those expected for air packs.

7.3.6 Changes in Lactic acid bacteria

During the initial day of storage lactic acid bacteria was 3.07 log cfu g^{-1}. Their count reached 6.7 log cfu g^{-1} for active oxygen scavenger packed samples at the end of study (Fig. 7.6). A similar result was also reported for yellow croaker (Li et al., 2013a). According to Nychas & Skandamis, (2005) modified atmosphere packing and vacuum packing favour the dominance of facultative anaerobic microbes including Lactic acid bacteria and *B. thermosphacta*. In the present study, Lactic acid bacteria being facultative anaerobic bacterial species, were found to dominate in active packed samples during the storage. A similar result was observed for seer fish steaks packed under reduced oxygen atmosphere (Mohan et al., 2010), mulltes stored under modified atmosphere pack (Pournis et al 2005), vacuum packed hake fillets (Fernandez-Saiz et al., 2013) vacuum packed salmon (Knochel, 1983), vacuum packed mackerel and Greenland halibut (Jeppensen & Huss, 1993). According to Korkeala & Bjorkroth (1997), *Lactobacillus spp* and *B thermosphacta* forms the major component of spoilage microflora in reduced oxygen concentrations. Lactic acid bacteria are considered as an important competitors of other spoilage related microbial groups under modified atmosphere conditions (Doulgeraki et al., 2012). Lactic acid bacteria inhibit the growth of other bacteria because of the formation of lactic acid and other bacteriocins and may contribute to their selective growth during spoilage (Li et al., 2011).

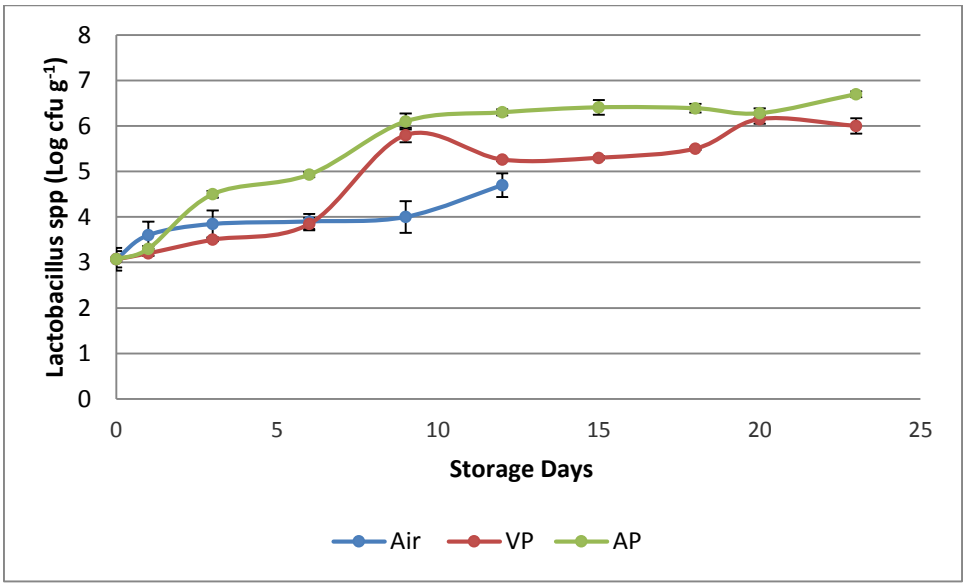


Fig 7.6. Changes in the *Lactobacillus spp*. count of yellowfin tuna chunks packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

7.3.7 Changes in *B thermosphacta*

Brochothrix thermosphacta is a gram positive psychrotrophic bacteria known as an important spoilage bacterium of various food matrices (Russo et al., 2006; Remenant et al., 2014). The initial count of *B thermosphacta* was 3 log cfu g^{-1} (Fig.7.7). At the end of the study, the content of *B thermosphacta* was 6.7, 7.1 and 6.2 log cfu g^{-1} for control air packs, vacuum packs and oxygen scavenger active packs respectively. In the present study, *B thermosphacta* was the dominat bacterial group next to Lactobacillus in active packed samples. In seafood packed under vacuum and modified atmosphere conditions, *B thermosphacta* can dominate the spoilage microflora at the expense of other genera like lactobacillus due to neutral pH, high water activity and high content of free amino acids and nucleotides (Jeyasekaran et al., 2006). The growth of *B thermosphacta* depends largely on the amount of available oxygen remaining in the package (Remenant et

al., 2014). In the present study, the dominance of Lactic acid bacteria in active packed samples can be explained in terms of the oxygen content in the packs. According to Remenant et al. (2014), *B thermosphacta* may become the dominant spoilage species when oxygen is present, but is displaced by Lactobacillus species under anaerobic conditions. Hence, the spoilage potential of *B thermosphacta* is influenced by the factors that control the level of residual oxygen inside the package. Both lactic acid bacteria and *B thermosphacta* are recognized as souring rather than putrefaction (Nychas et al., 2008). The dominance of gram positive bacteria such as *B. thermosphacta* and Lactic acid bacteria in active packs in this study can be considered as beneficial in the sense that by-products of *B thermosphacta* and Lactic acid bacteria are relatively bland compared to the typical foul spoilage odours produced by *Pseudomonas* and *Shewanella* (Miller et al., 1973; Dalgaard, 1995; Drosinos & Nychas,1997)

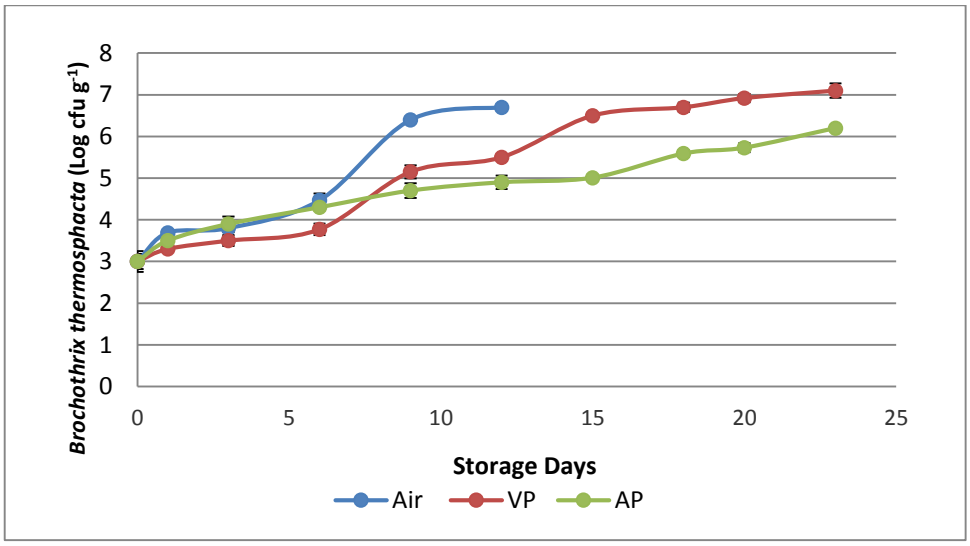


Fig 7.7.Changes in the total *Brochothrix thermosphacta* count of yellowfin tuna chunks packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 3, p<0.05

7.3.8 Changes in *Staphylococcus aureus* and *Escherichia coli*

Escherichia coli and *Staphylococcus aureus* were not observed in yellowfin tuna on the initial day of storage. It was not observed in any samples at the end of storage. It indicates the good microbial quality of the product.

7.3.9 *Clostridium botulinum* toxin production

In the present study, *C botulinum* toxin was not detected in any of the samples. In the present study, the storage temperature was maintained at 0-2^{0}C during the entire storage period. According to Graham et al. (1997), the temperature required for growth and toxin production of *C botulinum* is 3^{0}C. *C botulinum* toxin production was not reported in seer fish stored at reduced oxygen atmosphere during chilled storage (Mohan et al., 2010), seer fish steaks packed under modified atmosphere condition (Yesudhason et al., 2014) and modified atmosphere packed salmon and cat fish (Reddy et al., 1997). This may be due to the initial good quality of fish samples and proper maintenance of storage temperature. The most important safety concern of fish packed under reduced oxygen is the chances of occurrence of *Clostridium botulinum* and the subsequent botulinum toxin production.

7.3.10 Sensory analysis

The result of sensory analysis is presented in fig. 7.8. All samples showed a decreasing overall acceptability trend during storage. Active packed samples showed a significantly higher score ($p<0.05$) than the control air packed samples during storage. On the zero day of storage, the yellow fin tuna showed the characteristic odour, flavour and taste. Generally, the quality deterioration is characterized sensorially by the initial loss of fresh fish flavor and the spoilage of fish is characterized by the development of offensive fishy, rotten off odours and flavours (Gram & Huss, 1996; Gram et al., 1989).

The vacuum pack and control air pack were acceptable up to 12 and 9 days respectively. Whereas samples stored under active packaging retained good quality up to 20 days and were sensorily acceptable until the last day of storage. This could be due to suppression of spoilage microflora in the reduced oxygen content inside the active packs resulting in better preservation of all sensory quality parameters. Clingman & Hooper (1986) found a shelf life extension of 7 days for fresh fish products stored in vacuum packs. Özogul et al. (2006c) observed 12-15 days of acceptable shelf life for iced gutted trout and Tzikas et al. (2007) found 10 days shelf life for iced whole Mediterranean horse mackerel and 7 days for iced blue jacked mackerel. In the present study the microbial count of oxygen scavenger packed samples exceeded the microbial acceptable limit on 18th day. Even though the active packed samples were sensorily acceptable till the end of storage period, but the microbial count limited the shelf life of yellow fin tuna samples stored in active packs to 18 days.

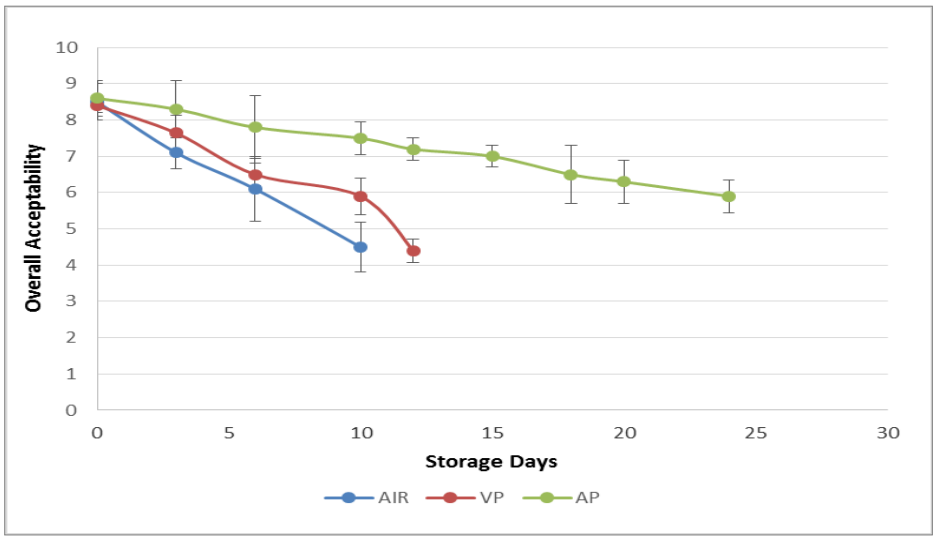


Fig. 7.8. The overall sensory acceptability of yellowfin tuna chunks packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with commercial oxygen scavengers) *Mean ± sd, n = 10, p<0.05

7.4 Conclusion

The application of oxygen scavengers were effective in extending the shelf life of yellow fin tuna up to 18 days compared to 9 and 12 days for control air and vacuum packed samples. The oxygen scavengers altered the condition inside the package and there was a shift in microflora from gram negative flora particularly H_2S producers in control air packs to gram-positive flora *B. thermosphacta* and *Lactobacillus spp.* in commercial oxygen scavenger packs. *C botulinum* toxin was not detected in any of the samples during the storage period. Oxygen scavengers used in the present study were very effective in reducing the oxygen concentration inside the packages. The shelf life was confirmed with microbial and sensory attributes in the case of vacuum and control air packs however, the shelf life estimated from sensory analysis differed from microbial analysis in the case of oxygen scavenger packed samples. Active packed samples were sensorily acceptable till the end of storage period, but the microbial count limited the shelf life of yellow fin tuna samples stored in commercial oxygen scavenger packs to 15-18 days.

Chapter -8

APPLICATION OF DUAL ACTION SACHETS FOR THE SHELF LIFE EXTENSION OF YELLOWFIN TUNA (*Thunnus albacares* Bonnaterre, 1788) DURING CHILLED STORAGE

Contents

Abstract

The effect of developed dual action sachets with CO_2 emission and O_2 scavenging capacity on the quality of yellowfin tuna was determined during chilled storage. It was then compared with samples packed with commercial oxygen scavenger, vacuum pack and air pack. All three developed sachets were able to reduce the oxygen concentration to less than 1% within 24 hours. The oxygen concentration was completely removed within second day and the oxygen free condition was maintained till the end of storage in the case of samples packed with developed sachets. Whereas the commercial oxygen scavengers reduced the oxygen content inside the packs to 0.01% within 24 hours of storage. All three chemical combinations emitted CO_2 during storage and maintaind the maximum CO_2 concentration till the end of storage. The application of developed dual action sachets SAI, SAIFC and SAFS* were able to extend the shelf life up to 20, 22 and 18 days of

storage. The samples packed with oxygen scavenger, vacuum and air packs showed a shelf life of 18, 12 and 9 days showing a significant increase of shelf life for samples packed with dual action sachets. The microbial quality, biogenic amines and K value correlated well in active packed samples including oxygen scavenger packs. *Lactobacillus spp* and *Brochothrix thermosphacta* formed the major micro flora in active packed samples.

8.1 Introduction

The demand for high quality fresh seafood has strengthened the search for minimal processing. One of the main developments in minimal processing is active packaging technology, which is designed to replace the use of chemical preservatives and other thermal technologies. Oxygen inside the package accelerates the oxidative deterioration of fish. It facilitates the growth of aerobic spilage microorganisms, off flavor, off odour and other oxidative deteriorative changes leading to nutritional loss and overall shelf life deterioration (Biji et al., 2015; Hogan & Kerry, 2008). Therefore, control of oxygen inside the package is important to limit the rate of oxidative deterioration in food. Oxygen scavengers are used to control the level of oxygen inside the package during storage and to protect from undesirable oxidative reactions (Brody et al., 2001).

High levels of CO_2 in the package also helps in extending the shelf life of muscle foods. Carbondioxide generators are mainly used in the package to inhibit microbial growth and it is especially effective against gram negative, aeriobic and psychrotrophic spoilage bacteria such as *Pseudomonas spp* (Gopal, 2007). It helps to increase the lag phase and generation time during the logarithmic growth phase (Coma, 2008). Packages alone with oxygen scavengers may cause the package collapse due to the partial vacuum

created by the removal of oxygen. In such cases, self-working systems which absorb oxygen and generate carbon dioxide are having great potential. Dual action sachets are designed to perform more than one active functions. For example, a combination of oxygen scavenger and carbon dioxide emitter, oxygen scavenger with antimicrobial emitter to significantly extend the shelf life of packaged foods. Hence the objective of the present study was to analyse the effect of dual action sachets with carbon dioxide emission and oxygen scavenging capacity on the shelf life extension of yellowfin tuna by monitoring physicochemical, microbial and sensory changes as a function of packaging treatment. It was then compared with commercial oxygen scavenger, vacuum and control air packs during chilled storage of yellowfin tuna.

8.2 Materials and methods

Yellowfin tuna procured from Fort Cochin fish landing center, Kerala, India, weighing approximately 7.5±1.5kg each with 71±4cm length and 20±1.5 cm width served as research material. Samples were washed with chilled potable water and brought to the laboratory in iced condition. Immediately after reaching the laboratory, samples were rinsed in chilled potable water and dressing was carried out manually. Yellowfin tuna chunks (100±10g) obtained were then divided into 6 lots and packed separately. First batch was packed as control air packs, the second lot was vacuum packed, third batch was packed with commercial oxygen scavengers. Other three batches (Table 8.1) were packed with developed active packaging sachet containing sodium bicarbonate:ascorbic acid:iron powder (SAI) (2:2:1); sodium bicarbonate:ascorbic acid:iron powder:ferric carbonate (SAIFC) (1:2:1:1); sodium bicarbonate:ascorbic acid:ferrous sulphate (SAFS) (2:2:1).

Table 8.1. Chemical combinations of dual action sachets used for the study

Sl No	Chemical combination	Symbol
12	2g sodium bicarbonate+ 2g ascorbic acid +1g iron powder	SAI
14	1g sodium bicarbonate+2g ascorbic acid + 1g iron powder + 1g ferric carbonate	SAIFC
16	2g sodium bicarbonate+ 2g ascorbic acid + 1g ferrous sulphate	SAFS

The packaging material used for the study was EVOH (Sealed air, India PVT Ltd, Bangalore, India) with 0.14 mm thickness having O_2 permeability of 60.8 cc/m^2/24hr and CO_2 permeability of 96.18 cc/m^2/24hr. Commercial O_2 scavenger sachets (Ageless® ZPT 200 EC O_2 absorber) with O_2 absorption capacity of 200 ml supplied by Sealed air (India) Pv Ltd, Bangalore, India were used for the study. One fish chunk (100±10g) was placed in all batches and sealed with sealing machine (Model No 405, Samarpan Fabarications Pvt Ltd, thane, India). Control air packs were packed without any active packing systems. Vacuum packs were sealed using a vacuum sealing machine (Vac Star S 210). Immediately after packing all packs were iced with flake ice in the ratio of 1:1 in insulated boxs and were kept in chill room maintained at 0-2^{0}C. Ice melt water was removed every day and fresh ice was filled to maintain the temperature. Three pouches from all batches were drawn at three day intervals and subjected to head space gas composition, physicochemical, microbial and sensory quality evaluation.

Plate 8.1 Yellowfin tuna chunk packed in EVOH control air pack

Plate 8.2 Yellowfin tuna chunk packed in EVOH vacuum pack

Plate 8.3 Yellowfin tuna chunk in EVOH pouch with commercial oxygen scavenger

Plate 8.4 Yellowfin tuna chunk in EVOH pouch with developed dual action sachet SAI

Plate 8.5 Yellowfin tuna chunk in EVOH pouch with developed dual action sachet SAIFC

Plate 8.6 Yellowfin tuna chunk in EVOH pouch with developed dual action sachet SAFS

Head space gas composition was measured with gas analyser (PBI Dansensor, Checkmate 9900, Ronnedevej, Denmark). TVB-N and TMA were estimated by the micro diffusion method (Conway, 1962). TBA value of the sample was estimated spectrophotometrically (Tarladgis et al., 1960). FFA was measured and expressed as mg % oleic acid (AOCS, 1989). PV was analysed and expressed as milli equivalent of O_2 kg^{-1} fat (AOCS, 1989). ATP breakdown products were determined according to the method of Ryder (1985) using High Performance Liquid Chromatography. A shimadzu prominence HPLC system consisting of LC20AD quaternary gradient pump, SPD 20A UV-VIS detector, Rheodyne 7725i manual injector and CBM-20A controller and C18 stainless steel colum was used for the study. Data acquisition and analysis was performed by LC solution software. The biogenic amine content of the sampeles was determinend using a rapid HPLC method described by Özogul et al., (2002b).

pH was determined according to APHA (1998) using a digital pH meter (Cyberscan 510, Eutech instruments, Singapore). The colour of the sample was measured with Hunter colorimeter (Hunter Lab colorimeter, MiniScan XE Plus Hunter Associates Lab inc., Reston, Virginia, USA). The redness index (a*/b*) of tuna meat was determined as described by Chen et al. (1997). Texture profile analysis (TPA) was measured with a Universal Testing Machine (Lloyd instruments LRX plus, UK). Water holding capacity was measured using a compression tester (Gaydon Compressor Tester, Model No 936, HE. Messer Ltd. London, UK). Water holding capacity was measured by dividing the volume of water released to the weight of sample. Drip loss was measured gravimetrically by taking the weight difference of the sample with and without exudate. The exudate was removed by draining and wiping the sample with filter paper.

Total mesophilic was determined by spread plate method using plate count agar (PCA) as per Townley & Lanier (1981)method. Enterobacteriaceae were enumerated on Violet Red Bile Glucose Agar (VRBGA, Oxoid, CM 485) as per Koutsoumanis & Nychas (1999). Hydrogen sulphide producing bacteria were enumerated on Peptone Iron Agar (PIA) (code 289100, BBL Difco) with added 1% salt (Gram et al., 1987). Black colonies formed by the production of H_2S were enumerated (Gennari & Campanini, 1991). Lactic acid bacteria were enumerated on MRS agar (de Man, Rogosa and Sharpe, Himedia (code M 641) supplemented with potassium sorbate as per Mossel (1987). Pure white colonies (2-3mm dia) were counted as *Lactobacillus spp*. For the enumeration of *Brochothrix thermosphacta*, Streptomycin Thallus Acetate Acetidione Agar (STAA) was used (Mead & adams, 1977). *Faecal streptococci* were enumerated on Kenner Faecal Agar (KF, BBL, Difco)

USFDA (1995). All red to pink colonies were counted as *Faecal Streptococci*. *Faecal streptococci* was determined only for fresh sample and for fish at the time of sensory rejection. *E coli* was enumerated on Tergitol 7 (T7) agar (Surendran et al., 2009). Sampling was carried out for fresh fish and for fish at the time of sensory rejection. Colonies with lime yellow, occasionally with rust brown center and yellow zone around were isolated and confirmed as *E coli* on EMB agar plates and by IMViC test. *Staphylococcus aureus* was enumerated on Baired Parker Agar (BP, Difco) (AOAC, 2000). Sampling was carried out for fresh fish and for fish at the time of sensory rejection. The black, convex colonies with clear margin zone were counted as *Staphylococcus aureus.* Typical colonies were isolated and confirmed by coagulase test. *Clostridium botulinum* toxin was detected by mouse bioassay as per the procedure described in US Food and Drug Administration Bacteriological Analytical Manual (USFDA, 2001). Sensory analysis of the samples was carried out by using a 9 point hedonic scale (annexure 1) as prescribed by Meilgaard et al., (1999).

Data are collected in triplicate and the results are presented as mean ± standard deviation. Experimental data were analyzed using two way analysis of variance. Marginal means of treatments and storage days were compared using tuky's post hoc test and least significant difference (lsd) was calculated for comparing interaction means at 5% level of significance. All the analysis were carried out using SAS 9.3.

8.3 Results and discussion

8.3.1 Changes in the head space gas compoisition

The changes in CO_2 and O_2 content in the head space of different packs during chilled storage are given in Fig 8.1 and 8.2. During storage it

was observed that the oxygen concentration of packs with commercial oxygen scavenger was reduced to 0.01% within 24 hours of storage. The developed sachets were able to reduce the oxygen concentration to less than 1% within 24 hours. The oxygen concentration was completely removed within second day and the oxygen free condition was maintained till the end of storage in the case of all three developed sachets. The oxygen concentration of vacuum and control air packs also showed a decreasing trend during storage. The decrease in oxygen concentration in control packs could be due to the microbial growth and by the oxidative deterioration of sample during storage. A similar result was observed by Torrieri et al. (2011) in blue fin tuna fillets stored under MAP.

The CO_2 concentration inside the package showed that the samples packed with developed sachet SAIFC was more efficient in attaining the maximum CO_2 concentration within 3 days of storage. The sachets maintained the CO_2 content inside the pouches till the end of storage period. All the three combinations did not show the similar CO_2 concentartion of above 85% observed during the standardization of chemical mixtures at room temperature. The reduction in CO_2 in the present study could be due to the dissolution of CO_2 during chilled storage. Milne et al. (2014) observed a similar result in modified atmosphere packed sammon fillets. The initial CO_2 content was 96% and it reduced to 45% on 8^{th} day of storage due to the dissolution of CO_2 into the water phase of salmon flesh. At day 11, insufficient gas remained in the headspace and the flexible packs remained 'snugged down' for the remainder of the trial. According to Gopal (2007), in modified atmospheric packs, partial vacuum is created inside the package as a result of dissolution of CO_2 into the product and removal of O_2. In such cases simultaneous release of CO_2 from CO_2 releasing sachets are desirable.

In the present study the developed sachets were effective in maintaining the carbon dioxide concentration inside the package throughout the study even if pack depression observed in packs indicating CO_2 dissolution in the product. The samples packed in control air packs and vacuum packs showed an increase in CO_2 concentration during storage. That may be due to the microbial growth during storage. The study indicated that the developed dual action sachets can be effectively used in place of modified atmosphere packaging with high levels of CO_2 and vacuum packaging respectively.

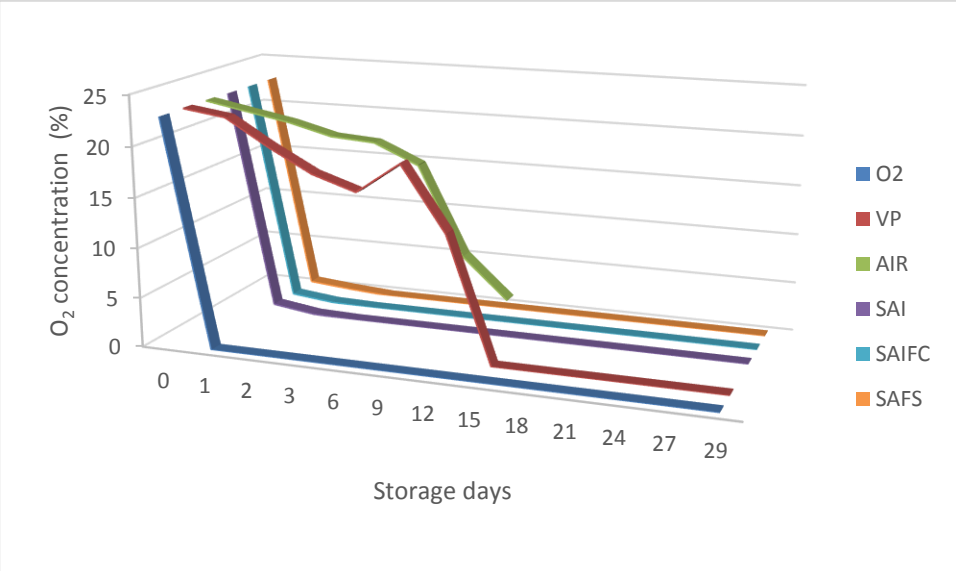


Fig. 8.1. Changes in head space gas composition (O_2)* of yellowfin tuna packs under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

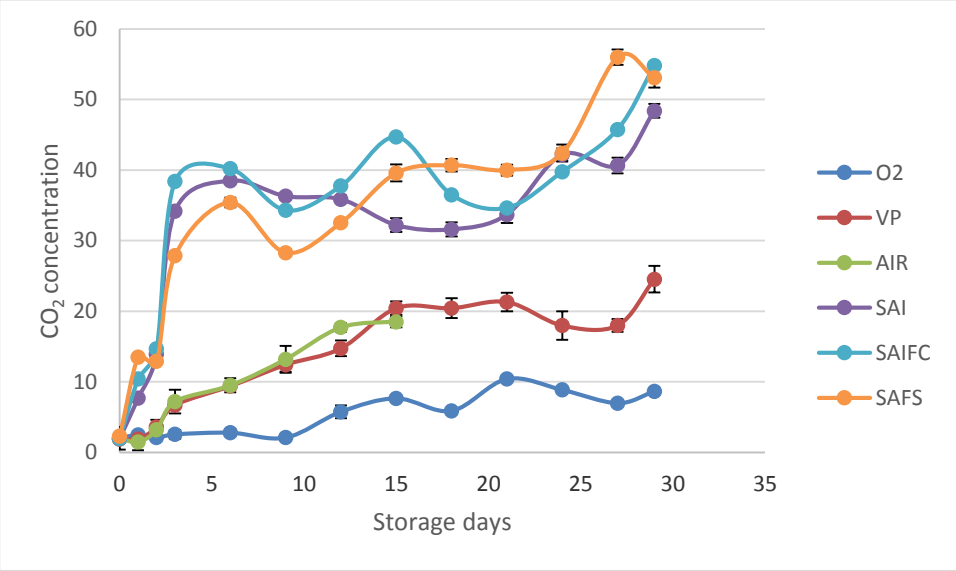


Fig. 8.2. Changes in head space gas composition (CO_2)* of yellowfin tuna packs under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.2 Changes in TVB-N

A number of compounds including dimethyl amine, trimethyl amine, ammonia or groups of compounds are considered as a chemical indicators for seafood spoilage. However, a single indicator could not be considered as a reliable index for a specific fish species. Total volatile base nitrogen in fish is produced mainly by the action of spoilage microbes on protein and non protein nitrogenous compounds such as amino acids and nucleotide catabolites (Cai et al., 2014). TVB-N is mainly composed of ammonia, methylamine, DMA, TMA, and it has been used to assess the quality of seafood during storage (Liu et al., 2013). Changes in TVB-N value of yellowfin tuna during storage is shown in Fig 8.3. The average initial TVB-N value observed in yellowfin tuna during the present study was 12.31 mg%. It increased gradually during storage in all samples. The control air and

vacuum packed samples reached the upper accepatability limit within 15 and 27 days of storage respectively. However, the oxygen scavenger packs and dual action sachet packed samples did not reached the upper acceptability limit till the end of storage period. A significant difference ($p<0.05$) in TVBN value was observed between control air packed samples and the other active packed samples during storage. Tukey's studentized range test showed that the marginal means of treatment were not significant for SAIFC and SAFS, but significantly ($p<0.05$) different from control air packed samples. Among the active packs, sachet with SAIFC showed least TVB-N value followed by SAI, SAFS and commercial oxygen scavenger packs. Such difference could be attributed to the preservative effect of active packaging. This effect could be attributed mainly to CO_2, owing to its antibacterial properties (Faber, 1991). TVB-N values of marine fishes were reported to increase linearly or curvy linearly during storage period as spoilage proceeds and a level of 30 mg N/100 g fish muscle is considered as the highest level of acceptability (Gökodlu et al., 1998). According to several authors, rise in this parameter is related to the activity of spoilage bacteria and endogenous enzymes, storage conditions, hygienic practices etc (Fernandez-Segovia et al., 2007; Özogul et al., 2009). In the present study, the active packed samples showed a lower TVB-N values that may be due to either reduced bacterial population or by decreased capacity of bacteria for oxidative deamination of nonprotein nitrogen compounds or both (Banks et al., 1980; Manju et al., 2007b). In the present study TVB-N values of active packed samples were well within the upper acceptability limit during the storage period, and hence it cannot be considered as a good index of spoilage.

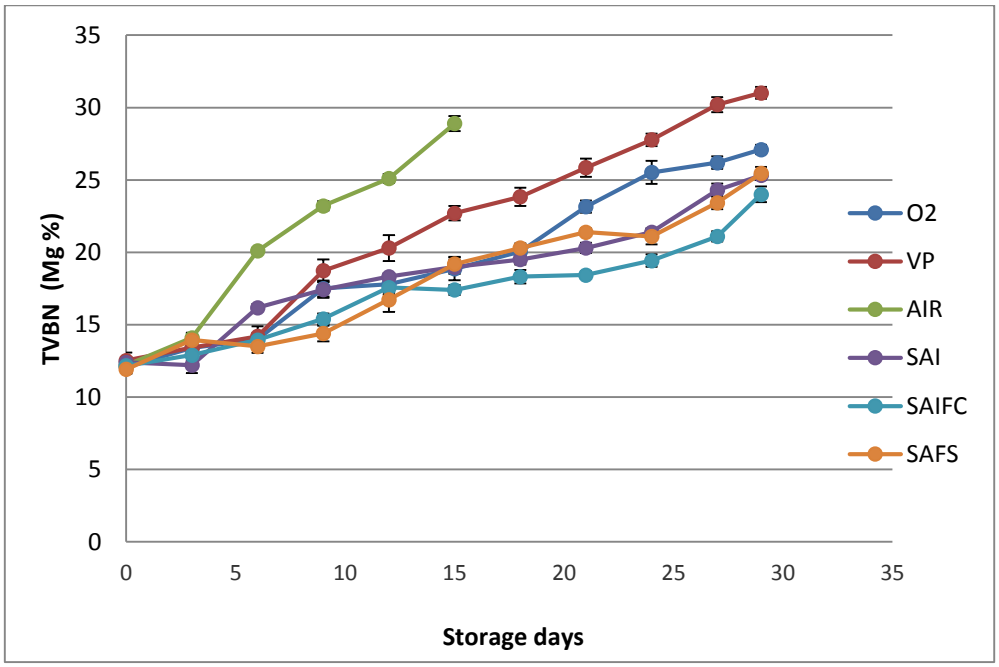


Fig. 8.3. Changes in TVB-N content of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.3 Changes in TMA

TMA is used as biochemical index to assess the keeping quality and shelf life of fish since it rapidly accumulates in the muscle under refrigerated storage. (Connel, 1990). TMA is produced by the decomposition of TMAO by the action of bacteria and enzymes. The pungent fishy smell of spoiled fish is associated with TMA and the spoilage microbes present in fish muscle (El Marrakchi et al., 1990). In the present study, the average initial TMA was 2.18 mg%, which indicates that the tuna used in the study was of good quality (Fig. 8.4). Tukey's studentized range test for TMA showed that

all the treatments were significantly ($p<0.05$) different from control air packed samples. Among treatments, samples packed with SAFS and commercial oxygen scavenger packs showed homogenous marginal mean. However, the marginal mean was significantly different from other treatments. During chilled storage TMA value showed an increasing trend ($p<0.05$) in all samples with storage time. A similar result was observed for fresh cold smoked salmon (Dondero et al., 2004). Cold smoked vacuum packed salmon (Leroi et al., 1998) stored under refrigerated conditions. In the present study, samples packed in air packs showed a value of 13.1mg% at the end of storage period. But none of the active packed samples led to values higher than the allowed legal limit of 5-10 mg TMA/100g (Sikorski et al., 1990b) or 15 mg TMA/100g (Sernapesca, 1996) till the end of storage. But it crossed the limit of 1 mg TMA/100g established by Kyrana et al. (1997) and Tejada & Huidobro (2002). This great variation in the acceptability level can be attributed to the fact that TMA values vary with species, season, storage conditions and bacterial as wel as the intrinsic enzymatic activities (Connel, 1990; Debevere & Boskou, 1996). The preservative effect of active packaging on yellowfin tuna is apparent. Fig 8.4 shows that TMA values recorded in active packed yellowfin tuna were significantly lower than those packed under air. Samples packed with dual action sachet SAIFC showed least TMA formation during storage. This can be attributed to the combined inhibitory effect of CO_2 after dissolution in both the aqueous and fatty phase of fish and the reduced oxygen content inside the packs in the case of samples packed with developed sachets. (Sivertsvik et al., 2002b; Goulas & Kontominas, 2007a). Fagan et al. (2004) observed no effect on TMA levels in mackerel and salmon portion packed in modified atmosphere packs. According to Bøknæs et al. (2000), the rise in TMA is related with the

growth of specific spoilage organism *P phosphoreum* which proliferates during iced storage.

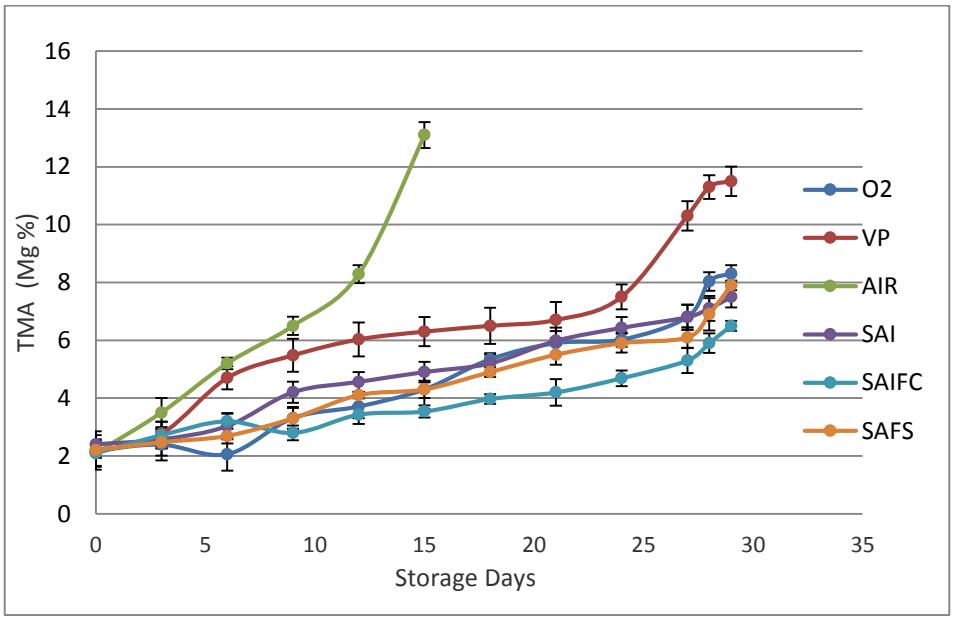


Fig. 8.4. Changes in TMA of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.4 Changes in FFA

Free fatty acid production is used to study the lipid hydrolysis and has been used to establish the degree of deterioration of food products. FFA is triacylglyceride product formed by either chemical or enzyme mediated hydrolysis (Barthet et al., 2008). In the present study, the FFA formation showed a progressive increase along with storage days in all cases (Fig. 8.5). Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. In

comparison with the initial material, samples packed in air packs showed a significant increase in FFA formation after 3rd day of storage. Eventhough the initial FFA content was lower, samples packed in active packs showed a significantly ($p<0.05$) slower rate of lipid hydrolysis during storage. Samples packed with commercial oxygen scavenger showed slower rate of FFA formation at the early days of storage however samples packed with developed sachet SAIFC sachet followed by SAI showed the least FFA value at the end of storage. This may be due to the protective action of developed sachets against lipid hydrolysis. The samples packed in vacuum packs reached a highest mean value of 15.9 mg% oleic acid at the end of storage period. Several authors studied formation of FFA in fish species such as sardine (Aubourg et al., 1997) horse mackerel (Aubourg, 2001b; Losada et al., 2005) blue whiting (Aubourg et al., 1998) and farmed turbot (Aubourg et al., 2005) during chilled storage. The increases rate of FFA formation in control samples during storage indicates enzymatic hydrolysis of the phospholipid (Koizumi et al., 1990). Formation of FFA itself does not relate with nutritional loss, but its assessment is considered important when considering the rancidity develomment and texture deterioration (Mackie, 1993; Sikorski & Lolakowska, 1994). Aubourg (2001a) explained the prooxidant effect of carboxyl group on the formation of free radicals by the decomposition of hydroperoxides. Being small size molecules, FFA have shown to undergo faster oxidation rate rather than bigger lipid classes significantly affecting the sensory quality of aquatic food products (Refsgaard et al., 2000; Labuza, 1971; Losada et al., 2007). Hence FFAs are sometimes quantified to establish whether or not a food product is organoleptically acceptable (Aubourg et al., 2007a,b; de Abreu et al., 2011b)

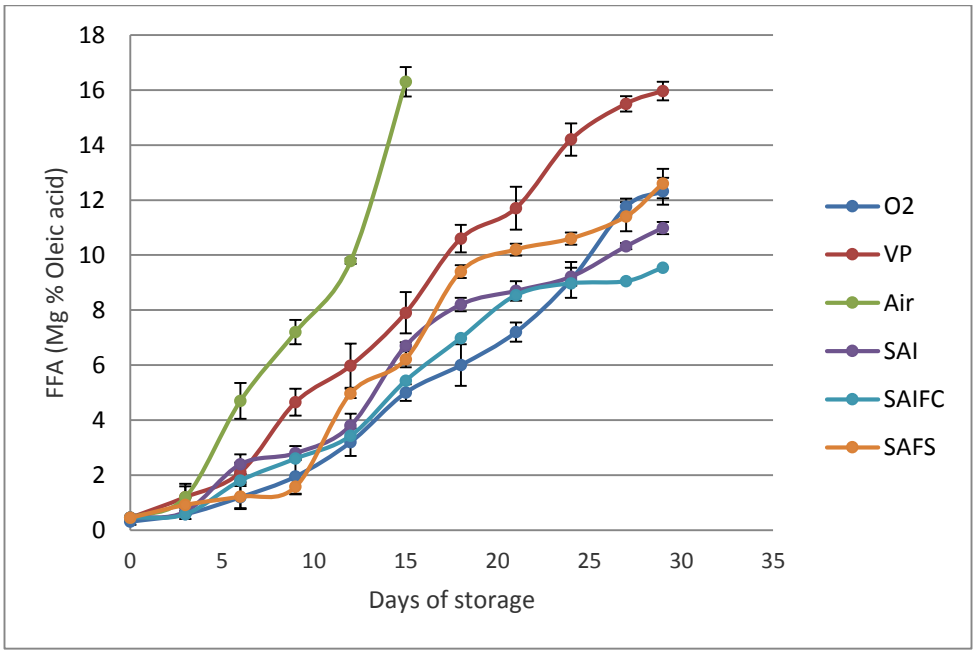


Fig. 8.5. Changes in FFA of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.5 Changes in PV

Peroxide value is used as an indicator for assessing the degree of lipid oxidation and indicates the amount of oxidized substances. These are usually hydroperoxides which produce iodine from potassium iodide under special conditions. Hydroperoxides are the primary products of autooxidation which themselves are odourless. However their decay leads to the formantion of a wide range of carbonyl compounds, hydrocarbons, furans and other products that contribute to the rancid taste of spoiled food (Yanishlieva & Marinova, 2001; Undeland et al., 1998). PV is affected by the age of raw material and oxidation of lipid during storage (AMT, 1997). Primary lipd

oxidation of yellowfin tuna muscle during chilled storage was measured by the changes in PV as given in Fig. 8.6. The average initial PV of yellowfin tuna sample was 0.45 milli equivalent/kg fat. In the present study, an increase in PV was observed in all samples during storage followed by a decrease towards the end of storage. Tukey's studentized range test showed that all treatments were significantly different ($p<0.05$) from control air packed samples. The rate of increase of PV value was significantly higher ($p<0.05$) for samples packed in control air. The samples packed in commercial oxygen scavenger sachets showed a slower rate of oxidation. That may be due to the protective action of oxygen scavenger against lipid oxidation. No significant difference in PV was found between samples packed with developed sachets. In the present study, after reaching a maximum value, the PV value started declining in all samples during storage. A similar result was observed in blue shark (de Abreu et al., 2011a) and Atlantic halibut (de Abreu et al., 2011b) during storage. The initial increase in PV occurs when the rate of formation of hydroperoxides higher than the rate of decomposition. A maximum value is reached and then decrease as a result of lower availability of substrate and the inability of peroxide molecules which leads to the lower speed of formation with respect to the rate of decomposition (de Abreu et al., 2011a,b). A similar result was observed for Nile tilapia (Yarnpakdee et al., 2012) oily monterey sardine (Pacheco-Aguilar et al., 2000) during chilled storage.

Lipid oxidation has been considered as a complex process where different kind of compounds are produced most of which are unstable. Such compounds are susceptible to degradation with the subsequent generation of low molecular weight compounds or reaction with other molecules present in the fish muscle. As a result of this the determination of each kind of

compound canot always provide an accurate method for the assessmet of quality loss in fish (García-Soto et al., 2013).

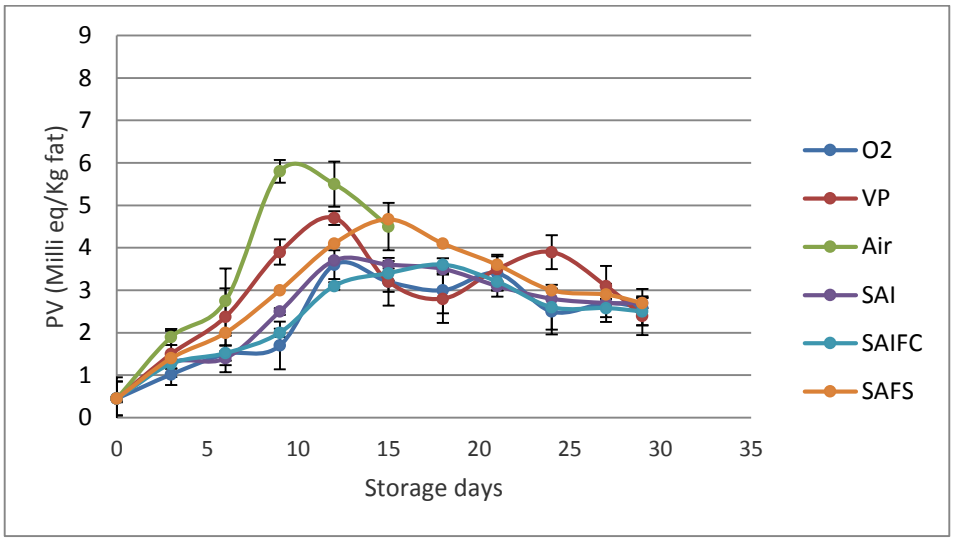


Fig. 8.6. Changes in PV of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.6 Changes in TBA value

Secondary lipid oxidation was also studied by the measurement of thiobarbituric acid value. TBA value is an index of lipid oxidation measuring malonaldehyde content (Goulas & Kontominas, 2007a). It is formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez et al., 1997). TBA, the secondary oxidation product of lipids of yellowfin tuna during chilled storage is presented in Fig. 8.7. The initial TBA value was 0.24 mg malonaldehyde/kg for yellowfin tuna muscle. A similar result was observed for trout fillets (Frangos et al., 2010), rainbow trout fillets (Neratzaki et al.,

2005) Atlantic halibut (de Abreu et al., 2011b). Samples packed in control air showed a significantly ($p<0.05$) higher value during storage. Samples packed in active packs were below the spoilage indices established at 2 or less mg malonaldehyde equivalent/kg muscle for TBA (Nishimoto et al., 1985) beyond which fish will normally develop an objectionable odour/taste (Goulas & Kontomunas, 2007b). The Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. The oxygen scavengers and dual action sachets significantly depressed the oxidative rancidity development ($P<0.05$) during storage and never reached the upper acceptability limit till the end of storage. The samples packed with commercial oxygen scavenger showed the least TBA value at the end of storage period followed by SAIFC, SAFS and SAI dual action sachets. However in Tukey grouping, the marginal mean for treatment showed that all treatments including vacuum pack, commercial oxygen scavenger pack and developed dual action sachets were homogenous in nature. This observation indicates the fact that oxygen scavenging considerably reduced oxidation of yellowfin tuna during storage. PUFA in fish oxidise rapidly in aerobic conditions (Stammen et al., 1990) hence exclusion of oxygen from the pack helps to maintain lipid stability during storage. Samples packed in vacuum reached the upper acceptability limit at the end of storage. According to Aubourg (1993), TBA values do not represent the actual lipid oxidation rate due to the interaction between malonaldehyde and amino acids, proteins, glucose and other fish constituents during storage to form polymers that decrease the quality of fish.

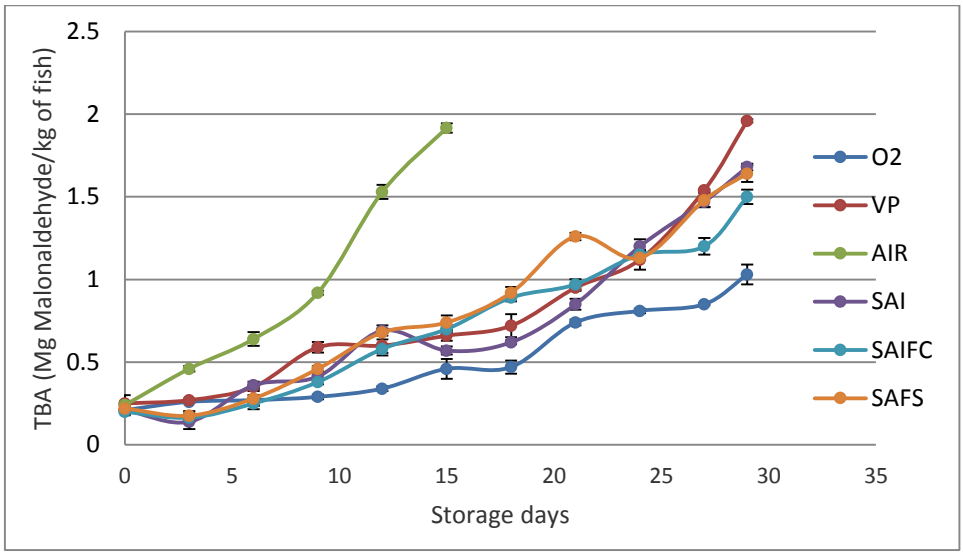


Fig. 8.7. Changes in TBA of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.7 Changes in pH

The postmortem pH fall refelects the glycogen store of the muscle. The pH reduction is caused by the formation of lactate by the breakdown of muscle glycogen (Love, 1988). It is important to avoid fast postmortem pH fall since it weakens the tissues between the muscle blocks and become separated and gaping takes place (Robb & Kestin, 2002). The average pH of yellowfin tuna meat during the initial day of storage was 6.15 (Fig 8.8). An initial pH value of 6.65 pH was observed for yellow grouper (Li et al., 2011), 6.6 for channel cat fish (Silva et al., 1993), 6.24 for sardine (El Marrakchi et al., 1990), 5.89 for yellowfin tuna (Emborg et al., 2005), 6.43 for Cazon fish (Ocaño-Higuera et al., 2009), 6.35 for pink salmon (Barnett et

al., 1991). Variations amoungst the initial pH values in different species may be due to season, diet, level of activity or stress during capture and type of muscle (Ocaño-Higuera et al., 2009). Post mortem pH values can vary from 6.6 to 7.1 depending on species, season, diet, lavel of activity and other factors (Li et al., 2011). In the present study, pH value decreased in all samples during chilled storage. Tukey's studentized range test showed that there was no significant difference ($p<0.05$) in the marginal mean of treatments between control air, developed sachets with SAIFC and SAFS. Treatments vacuum pack and commercial oxygen scavenger packs were also homogenous in nature in Tukey's grouping. Samples packed with SAFS showed least pH value at the end of storage days. The other dual action sachets also showed a similar decreasing trend of pH during storage. This may be due to the dissolution of CO_2 and formation of carbonic acid during chilled storage. A decrease in pH was observed for seafood processed in CO_2 (Genigeorgis, 1985). According to Sivertsvik et al. (2002a), fish packed in rich CO_2 atmosphere leads to the dissolution of CO_2 into the product and carbonic acid is produced. The ionization of this carbonic acid leads to the reduction of pH in seafood. In the present study, pH value of all samples decreased during chilled storage. Water holding capacity is closely related to pH of fish muscle (Rustad, 1992). According to Sikorski et al. (1990b) lactic acid genetared in anoxic conditions from glycogen into lactic acid is the principal factor in lowering postmortem pH in fish.

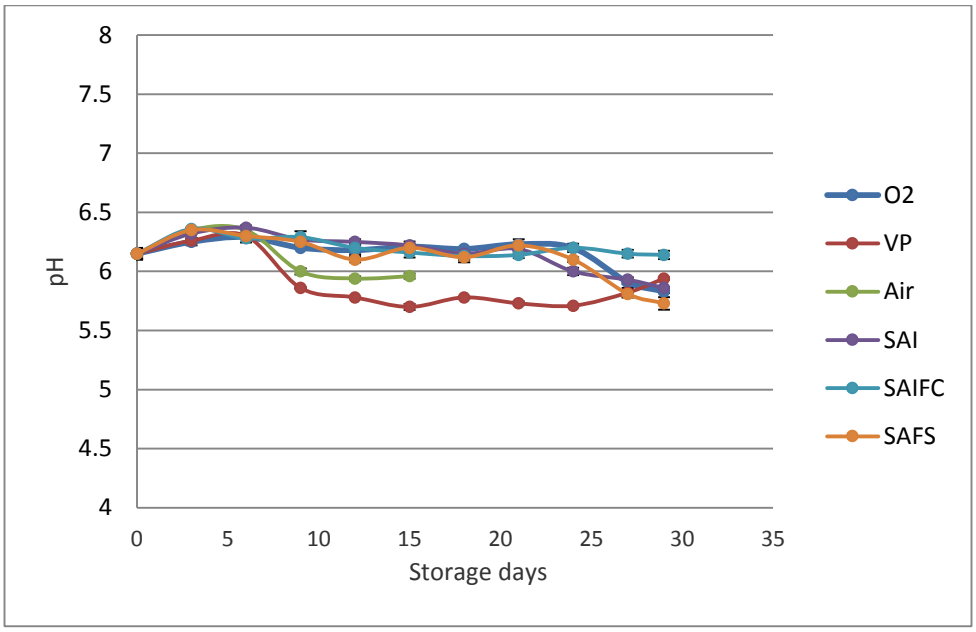


Fig. 8.8. Changes in pH of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.8 Changes in L*

Colour is an important quality attribute since it directely influences the purchasing behavior of a consumer. In the CIE L*, a* and b* system, L* represents lightness on a scale from 0 to 100 ie from black to pure white. a* represents (+) red or (-) green and b* denotes (+) yellow or (-) blue (Pérez-Alvarez & Fernández-López, 2000). According to Haard (1992a,b), the initial colour of seafood change with storage time in ice. The values of lightness (L*), redness (a*) and yellowness (b*) of samples packed under various atmospheric conditions during chilled storage in the present study is given in Fig. 8.9. a,b and c respectively. The average initial L* value of

yellowfin tuna meat was 33.8. Lightness (L*) value remain rather stable during storage. Tukey's studentized range test showed that all treantments were significantly ($p<0.05$) different from control air packed samples. The marginal mean for treatments showed that developed sachets SAI and SAFS were homogenous in nature, but significantly different from other treatments including SAIFC, vacuum and commercial oxygen scavenger packs. A similar result was also observed in *Thunnus obesus* (Muela et al., 2014; Imamura et al., 2012), salmon (Gimenez et al., 2005) sea bass fillets (Provincial et al., 2010) mackerel, whiting and salmon (Fagan et al., 2004) packed under modified atmosphere conditions. Gerdes & Valdez (1991) observed a decreasing L* values under modified atmosphere packs during chilled storage of red snapper.

8.3.9 Changes in a*

In the present study, a* value was influenced during storage. A significant difference ($P<0.05$) was observed between the samples packed under air and active packed samples as a function of time. Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. The samples packed with developed dual action sachets and active oxygen scavengers showed significantly ($P<0.05$) slower rate of a* reduction during storage. Tukey's grouping showed that the marginal mean of SAI and SAIFC were homogenous. However significantly different from other treatments. A similar result was also observed by Tanaka et al. (1996) for tuna fillets, Torrieri et al. (2011) for Bluefin tuna stored at MAP showing CO_2 effective in keeping fresh red colour. According to Imamura et al. (2012) a general decrease in a* value occurs due to the formation of metmygoglobin from oxymyoglobin. This occurs when fish is cut up, oxygen comes in contact

with myoglobin in the exposed tuna meat surface. The oxygen is absorbed and reacts with the myoglobin to form bright red pigment (oxymyoglobin) which brings about the attractive red colour of fresh tuna meat (Tajima & Shikama, 1987). Hence it is very important to remove oxygen from the package as early as possible to prevent the formation of metmyoglobin. Discolouration in air packed samples could be related to the formation of metmyoglobin and its accumulation on the surface. The stability of a* value in oxygen free atmosphere can also coupled with the muscle chemistry, that may not be capable of further oxygen consumption coupled with the reduction of ferric to ferrous iron.

8.3.10 Changes in b*

Changes in b* value during chilled storage is given in Fig 8.9.c. In the present study, an increase in b* value was observed in all samples during chilled storage. Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. The marginal mean for treatments showed that among treatments, SAFS and SAIFC were homogenous in nature. Treatments SAI and commercial oxygen scavenger were also homogenous in nature, but significantly different from control air and vacuum packed samples. The samples packed in control air packs showed the maximum b* value at the end of storage. Increase of b*indicates more yellow colour of fish muscle during storage. That may be due to the pigments derived from lipid oxidation, haeme protein oxidation and other browning reactions (Haard, 1992a). Haeme proteins once oxidized to metmyoglobin, can give a brown yellowish appearance to red muscle thus increasing the b* value (Kristinsson & Demir, 2003; Concollato et al, 2014). Tironi et al. (2010) also observed an increase

in b* value in seabass during frozen storage. In the present study, even though the samples showed an increasing trend during storage, but they didn't find any statistical difference between treatments during storage. A similar result was also observed for Bluefin tuna (Torrieri et al. 2011) packed in air and MAP during chilled storage. Hence, the experimental results showed that a* and b* values are important quality indices to study the quality of yellowfin tuna during active packaging.

8.3.11 Changes in redness index (a*/b*)

Redness index is used to evaluate the discolouration in tuna meat during storage (Lee et al., 2003). It is used as an index of apparent changes in redness (Chen et al., 1997). Redness index is shown in Fig. 8.9.d. Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. In the present study, the samples showed a decreasing trend in redness index during storage irrespective of storage atmosphere. However the samples packed in air showed a significantly ($P<0.05$) lower redness index at the end of storage period. The active packed samples showed lower rate of reduction of redness index during storage period. The samples packed with commercial oxygen scavenger showed the maximum redness index at the end of storage. Chaijan et al. (2005) observed a decrease in redness index in sardine and mackerel during chilled storage. Decrease in redness index is associated with darkening of meats resulting from the formation of met myoglobin (Chaijan et al., 2005). According to Faustman et al. (1992) the saturation of red colour in meat is directely related to myoglobin concentration. Dark discolouration in meat is directely associated with the total pigment concentration (Fleming et al., 1991). Hence, changes in redness index can be used to assess the freshness of yellow fin tun meat.

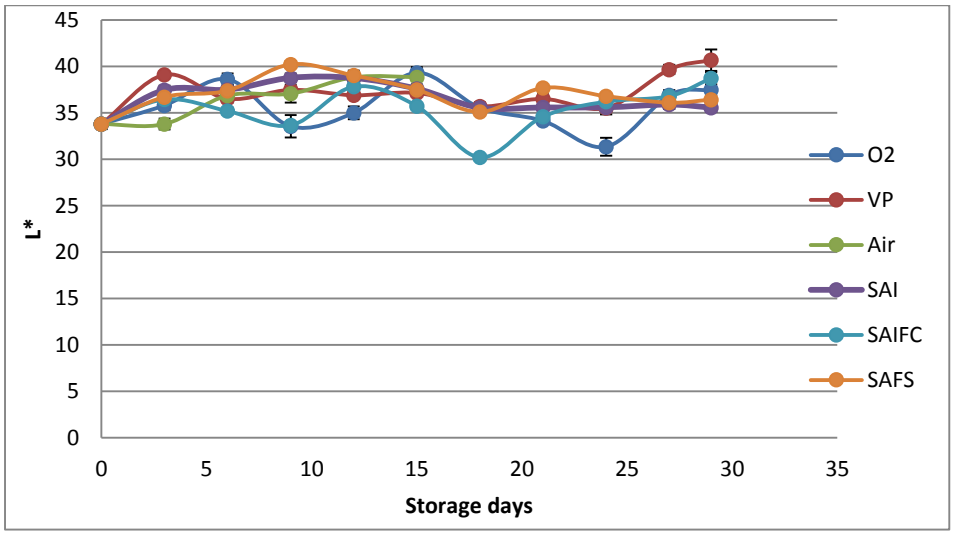


Fig. 8.9.a. Changes in L* of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

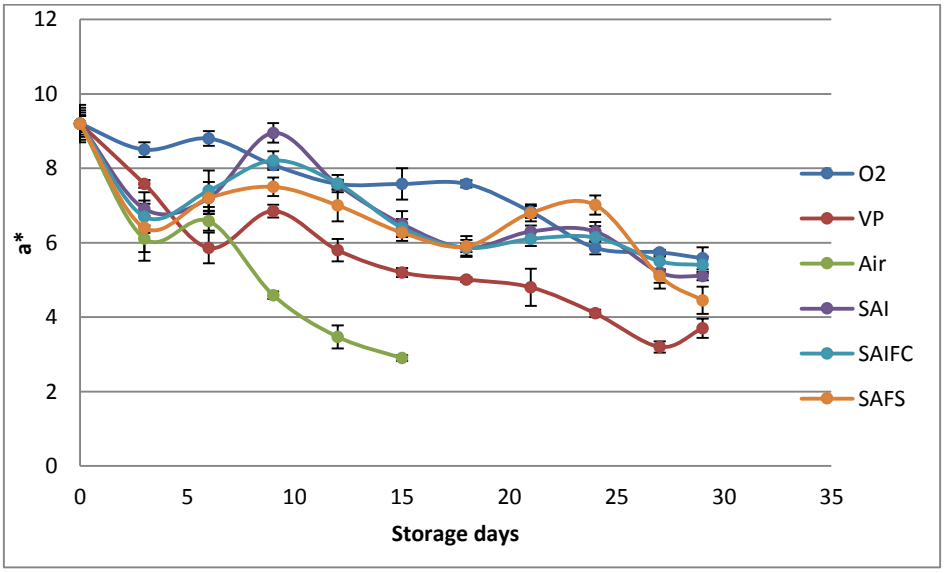


Fig. 8.9.b. Changes in a* of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

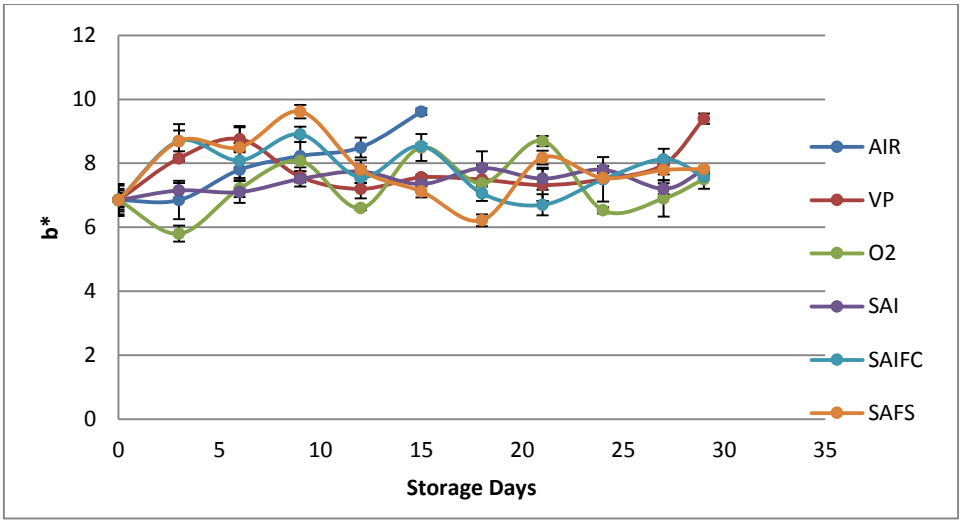


Fig. 8.9.c. Changes in b* of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

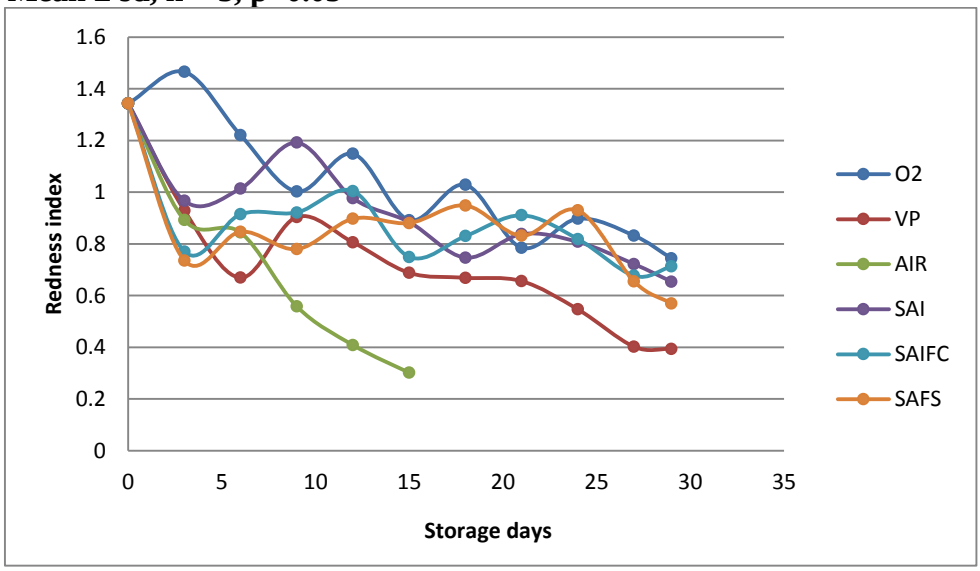


Fig. 8.9.d. Changes in redness index of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± std, n = 3, p<0.05

8.3.12 Changes in sensory evaluation score

The sensory changes occur in appearance, colour, odour, taste, and texture during storage. Sensory evaluation of raw and cooked fish is the most convenient and successful method for fish freshness evaluation (Olafsdottir et al., 1997) since consumer is the ultimate judge of quality (Connell, 1986). The orveral sensory score of yellowfin tuna during chilled storage are shown in Fig. 8.10. Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. All the active packed samples showed a significantly higher overall acceptability score during the storage period. Initially fish had fresh seaweady smell with firm texture and colour. Progressive quality loss was observed during storage in all samples. The changes were mainly with regard to the difference in taste, texture, flavor and colour. The quality attributes were stable even up to five days in the case of control air packed samples, and ten days for vacuum packed samples. The active packed samples were stable even up to 15 days. The samples packed with active oxygen scavengers became sour in taste at the end of storage study. The samples packed in control air packs were sensorily rejected on the 10^{th} day of chilled storage. The samples were found to be unacceptable on 12^{th} day of storage in the case of control vacuum packs. Samples packed with commercial oxygen scavenger and dual action sachets SAI, SAIFC and SAFS were acceptable till the end of storage since the samples did not develope any foul smell and they maintained the appearance till the end of storage. The results were not in accordance with the microbial count of active packed samples observed in the present study. In active packed samples, the colour, flavor and smell was not significantly different with time during storage period. The chemical and microbial quality parameters were supported by the sensory evaluation results

of samples packed in control air and vacuum. Özogul et al. (2004) observed 12 days of shelf life for sardine stored at MAP, 9 days in VP and 3 days for air packs during chilled storage. Özogul et al. (2000) observed 10 days of shelf life for herring stored in MAP and 8 days in VP chilled storage. Clingman & Hooper (1986) observed an overall increase of 7 days of shelf life for vacuum packed fish over aerobically stored fish. Cann et al. (1983) observed that sensory evaluation limited the shelf life of herring to 8 days in air packs wheras 13 days of shelf life was obtained for herrings packed under vacuum. In the present study, the sensory evaluation results were in accordance to the results of microbial counts in the case of vacuum and air packed samples. However, the samples packed with active packs were not in agreement with the microbial results since the active packed samples reached the upper microbial acceptability limit on 18, 20, 22 and 18 days for commercial oxygen scavenger packs and developed dual action sachets with SAI, SAIFC, and SAFS respectively. Hence shelf life of active packed samplels were fixed based on microbial result.

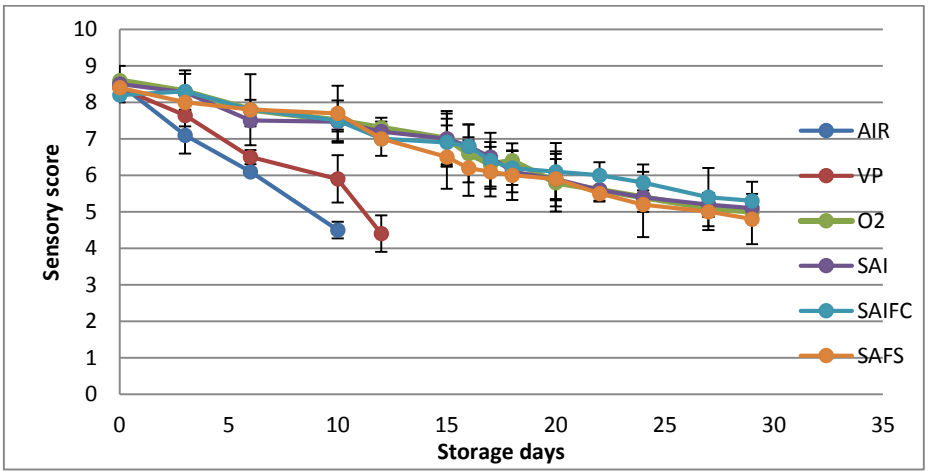


Fig. 8.10. Changes in sensory score of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± std, n = 3, $p<0.05$

8.3.13 Changes in aerobic mesophilic bacterial count

The postmortem degradation of seafood is caused by endogenous enzymes and microorganisms along with contaminants during processing (Barros-Velázquez et al., 2008). In order to increase the shelf life the initial microbial counts must be reduced using strict hygienic conditions in the industry (Fernandez et al., 2009). The average initial aerobic mesophilic count of yellow fin tuna was 4.6 log cfu/gram which indicates the good quality of the tuna sample used for the study. A similar result was also observed for yellow fin tuna dark muscle (Sanchez-Zapata et al., 2011), fresh yellowfin funa muscle (Kristinsson et al., 2008) sword fish (Pantazi et al., 2008), seer fish (Mohan et al., 2010), mackerel and salmon (Fagan et al., 2004). The changes in mesophilic bacterial count of yellowfin tuna during chilled storage is presented in Fig. 8.11. The mesophilic bacterial count showed an increasing trend irrespective of the packaging atmosphere however the count was significantly higher ($p<0.05$) in control air packs. Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. The marginal mean of treatments showed that treatments SAIFC and SAI were homogenous, but significantly different from other treatments. Initial lag phase was not observed for samples packed with control air and vacuum packed samples. The mesophilic count crossed the value of 7 log cfu/g which is considered as the upper acceptability limit for fresh water and marine species (ICMSF, 1986) on the 9^{th} day of storage indicating a shelf life of 6-9 days for control samples. The vacuum, oxygen scavenger packs, and dual action sachets SAI, SAIFC and SAFS reached the upper acceptability limit on 12^{th}, 18^{th}, 20^{th}, 22^{rd} and 18^{th} day indicating a shelf life of 9-12, 15-18, 18-20, 20-22 and 15-18 days respectively. Samples packed in commercial oxygen scavenger packs

and developed dual action sachets SAI, SAIFC and SAFS showed an initial 3 days lag period. The lag phase observed in the present study could be attributed to the inhibitory effect of low oxygen content and the inhibitory action of carbon dioxide on the growth of microorganisms. A similar lag phase was observed in seer fish packed with commercial oxygen scavenger (Mohan et al., 2010), and also under MAP (Yesudhason, 2007). Huss (1972) indicated that CO_2 exerts a selective inhibitory action on microbial growth. The antibacterial activity of CO_2 against many spoilage bacteria was reported earlier (Ruíz-Capillas & Moral, 2002; Devlieghere et al., 1998). CO_2 is regarded as bacteriostatic rather than bacteriocidal agent (Eyles et al., 1993). Guizani et al. (2005) reported that aerobic mesophilic bacteria and aerobic psychrotropic bacteria dominate the microbiota in yellowfin tuna muscle. Sivertsvik et al. (2002a) explained the four mechanisms responsible for the bacteriostatic function on bacteria as being alteration of cell membrane functioning, direct inhibition of enzymes or decrease in the rate of enzyme reactions, penetration of the bacterial membranes leading to intracellular pH change and direct changes in the physico chemical properties of proteins. To achieve the antimicrobial benefits, the storage temperature of MAP should be as low as possible, since the solubility of CO_2 depends on the temperature. Solubility increases with decrease in temperature (Daniels et al., 1985; Özogul et al., 2004). The concentration of dissolved CO_2 in flesh is influenced by gas: product volume ratio (G/P) and storage temperature (Devlieghere et al., 1998).

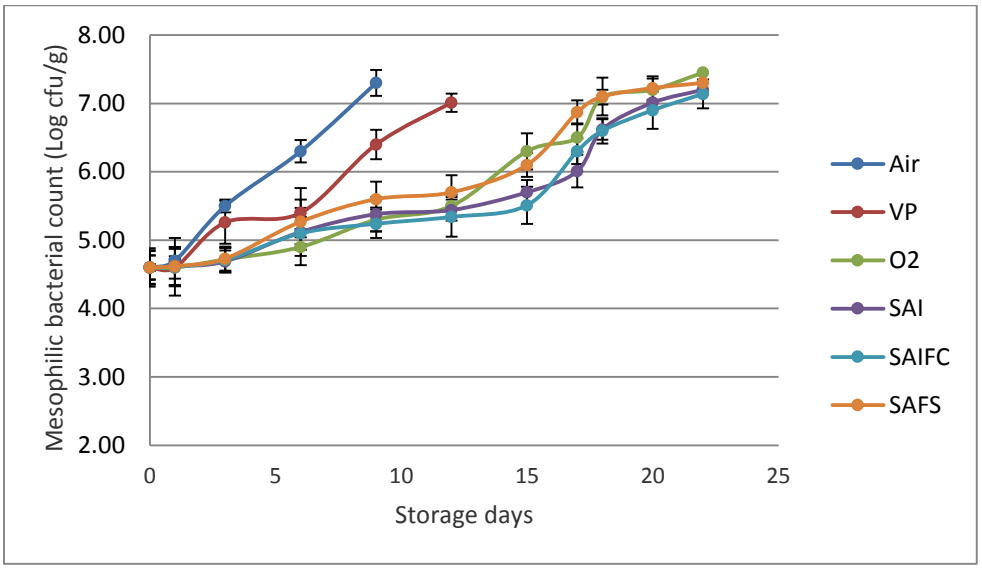


Fig. 8.11. Changes in mesophilic bacterial count of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.14 Changes in enterobacteriaceae.

The contribution of Enterobacteriaceae to the microbial flora of fish and its spoilage potential must be taken into consideration especially in the case of polluted water or delay in chilling after catch (Lindberg et al., 1998). In the present study, the average initial count of Enterobacteriaceae was 1.9 log cfu/g. This result is in agreement with the result reported for sword fish (Pantazi et al., 2008), gilthead sea bream (Tejada & Huidobro, 2002). The enterobacteriaceae count increased significantly ($p<0.05$) during storage in the case of control air and vacuum packed samples (Fig. 8.12). The average initial enterobacteriaceae count increased from 1.9 log cfu/g to 4 log cfu/g at the end of storage period in the case of control air packed samples. In samples packed with active oxygen scavenger packs,

enterobacteriaceae count gradually increased to 3.0 log cfu/g on day 9, thereafter a decreasing trend was noticed and the count was 1.9 log cfu/g at the end of storage period. In samples packed with dual action sachets SAI and SAFS also showed a similar trend. An increase in count to 3.6 log cfu/g and 3.7 log cfu/g was noticed on day 9 in SAI and SAFS respectively and at the end of storage, the count was <3.0 log cfu/g. The samples packed with dual action scahet SAIFC, though showed a similar trend during storage, the maximum count of 3.64 log cgu/g was noticed on day 12 and thereafter, it decreased to <3.0 log cfu/g at the end of storage. The samples packed with oxygen scavenger packs showed the least enterobacteriaceae count at the end of storage. Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. The marginal mean for treatments showed that the samples packed with SAIFC and SAFS were homogenous in nature. Treatments SAI and SAFS were also homogenous in nature, but significantly different from control air packed samples.

A similar initial enterobacteriaceae count was observed for sword fish (Pantazi et al., 2008), black pomfret (Manju, 2005), and temperate marine fish (Koutsoumanis & Nychas, 1999). A higher enterobacteriaceae count was observed for ice stored meagre fillets (Hernandez et al., 2009). Enterobacteriaceae being psychrotolerant are capable of growing at refrigerated temperatures. However they cannot compete well with gram negative spoilers (ICMSF, 1998). The results of the study indicates the bacterial quality of yellowfin tuna stored under chilled condition

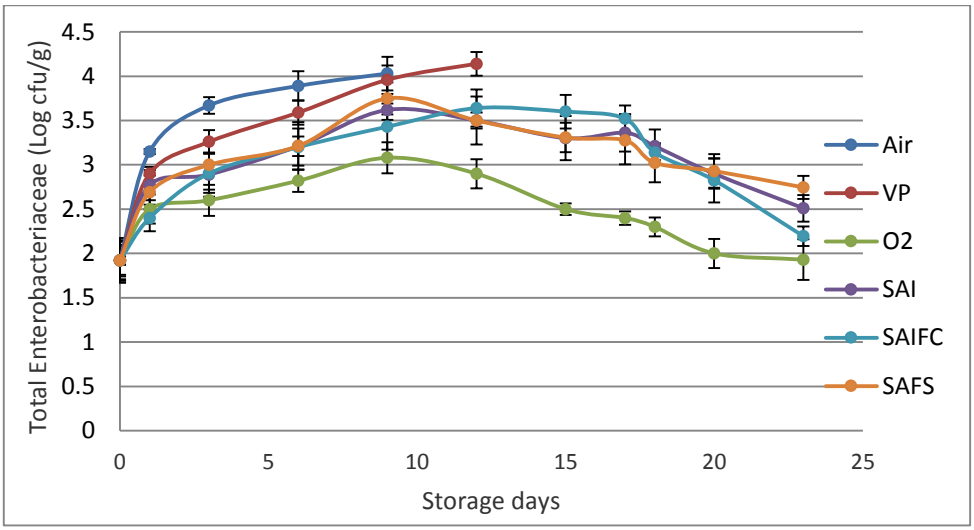


Fig. 8.12. Changes in Enterobacteriaceae count of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.15 Changes in *Brochothrix thermosphacta*

The counts of *B thermosphacta* showed an increasing trend throughout storage period. Tukey's studentized range test showed that all the treatments were significantly different from control air packed samples. Tukey's grouping showed that the marginal mean of SAIFC and SAI were homogenous in nature. The control air packed samples showed a significantly higher ($p<0.05$) count compared to other active packed samples (Fig. 8.13.). As a prominent spoilage microorganism, *B spermosphacta* is able to grow at chilled temperatures and high salt concentrations with (~ 10%) with its proliferation depends on the amount of oxygen present (Gribble et al 2014). There was no lag phase observed for samples packed in control air packs wheras a lag phase of 3 days was observed for samples packed with vacuum,

commercial oxygen scavenger and dual action sachets SAI, SAIFC and SAFS indicating the effect of altered environment on the initial bacterial growth. At the end of the study the count of *B thermosphacta* was 6.2, 5.8, 6.2, 6.4 6.5, and 6.6 log cfu/g for samples packed in control air, vacuum, oxygen scavenger, developed dual action sachets SAI, SAIFC and SAFS respectively. In the present study, *B thermosphacta* was the dominat microbial group after *Lactobacillus* in active packed samples. A similar result was also observed in carbon dioxide enriched atmosphere packed salmon steaks (De la Hoz et al., 2000), oxygen scavenger packed cobia fish steaks (Remya et al 2017) and hake steaks stored in CO_2 enriched atmosphere (Ordonez et al., 2000). *B thermosphacta* produce mainly lactic acid and ethanol under anaerobic conditions (Hitchener et al., 1979) and under anaerobic conditions, it produces acetoin, acetic, iso butyric, 2 methylbutyric and isovaleric acids and 3 methybutanol (Dainty & Hibbard, 1980; Mohan et al., 2010).

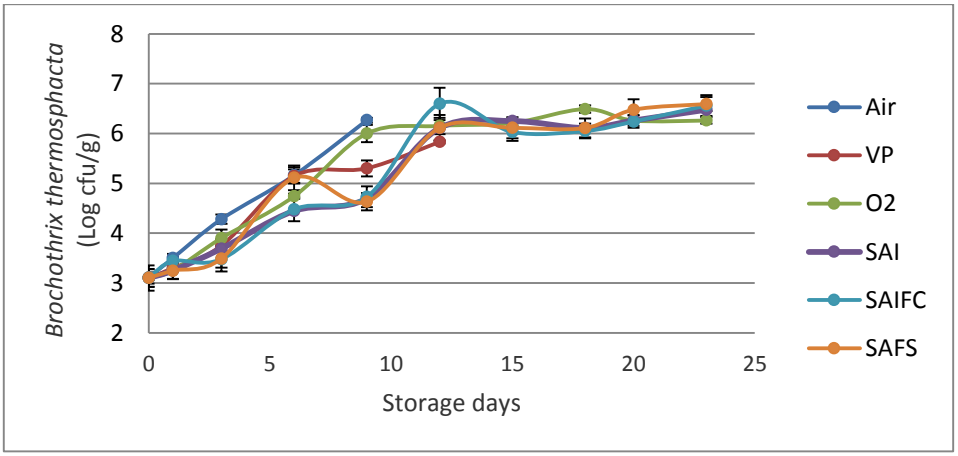


Fig. 8.13. Changes in *Brochothrix thermosphacta* count of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.16 Changes in H_2S producers

Counts of H_2S producing bacteria have been used as spoilage indicators of seafood prodcts (Gram & Dalgaard, 2002). Changes in H_2S producers of yellowfin tuna during storage is presented in Fig. 8.14. The initial H_2S producing bacterial count was 4.3log cfu/g. A similar initial count was observed in seer fish by Yesudhason (2007). A lower initial count of 2.3 log cfu/g was observed in sword fish (Pentazi et al., 2008). Tukey's studentized range test showed that all the treatments were significantly ($p<0.05$) different from control air packed samples. Tukey's grouping for marginal mean showed that treaments SAI and SAFS were homogenous in nature. Treatment SAI and SAIFC were also homogenous in nature, but significantly different from vacuum, commercial oxygen scavenger and air packed samples. In the present study the H_2S producers increased after a lag phase of 3 days in control air and 6 days in vacuum packed samples to give final population of 6.7 log cfu/g and 5.5 log cfu/g in control air and vacuum packed samples respectively. Samples packed with commercial oxygen scavenger packs showed a lag phase of 9 days, whereas counts fluctuated between 4.3log cfu/g to 4.6 log cfu/g in the case of SAI and 4.3 log cfu/g to 4.9 log cfu/g in the case of SAIFC and SAFS. The increased lag phase observed in dual action sachets could be attributed due to the effect of carbondioxide released from dual action sachets during storage. The counts thereafter increased to 6.2 log cfu/g, 5.4 log cfu/g, 5.2 log cfu/g and 5.6 log cfu/g in commercial oxygen scavenger packs, SAI, SAIFC and SAFS packs respectively at the end of storage. In developed dual action sachets, SAI, SAIFC and SAFS, the count slowly increased and reached 5.4 log cfu/g, 5.2 log cfu/g and 5.6 log cfu/g respectively at the end of storage period. H_2S producing bacteria were significantly ($P<0.05$) lower in developed sachets.

H_2S producing bacteria was found to be one of the dominating micro flora in control air packs in the present study. Earlier investigations showed that CO_2 has an inhibitory effect on H_2S producing bacteria (Huss, 1988).

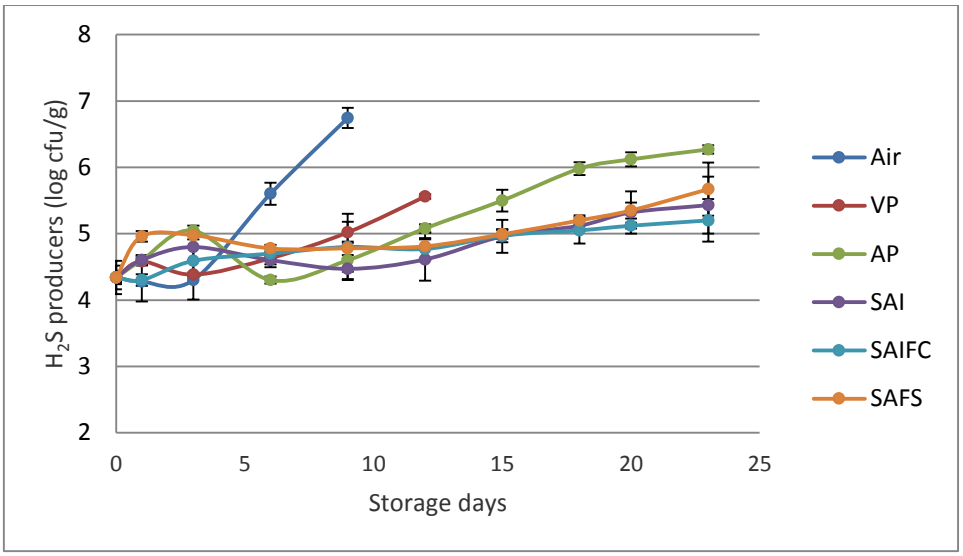


Fig. 8.14. Changes in H_2S producesr count of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.17 Changes in Lactic acid bacteria (LAB)

LAB being facultative anaerobic bacterial species were found to be dominating in samples packed with developed dual action sachets and commercial oxygen scavenger packs in the present study. Tukey's studentized range test showed that all treatments were significantly ($p<0.05$) different from control air packed samples. Tukey's grouping for marginal mean showed that samples packed with commercial oxygen scavenger and SAFS were homogenous in nature. Treatment SAIFC and commercial oxygen scavenger were also homogenous in nature. The yellowfin tuna showed an initial LAB of 3.1 log cfu/g (Fig 8.15). Pantazi et al., (2008)

observed an initial LAB count of 3.7 log cfu/g in sword fish. Lactic acid bacteria dominated in yellowfin tuna during chilled storage in vacuum and other active packed samples irrespective of active packaging treatments. The lactic acid bacterial count reached 6.1, 6.9, 6.8, 6.6 and 6.7 log cfu per gram in vacuum, oxygen scavenger, and developed dual action sachets SAI, SAIFC, and SAFS respectively at the end of the study. The results in the present study are in good agreement with those reported for oregano essential oil treated vacuum packed Mediterranean octopus (Atrea et al., 2009). A similar result was observed in vacuum packed cold smoked refrigerated salmon (Donderoa et al., 2004; Leroi et al., 1996,1998), vacuum packed salmon (Jeppensen & Huss (1993), sword fish stored in CO_2 enriched atmospheres (Lannelongue et al., 1982; Kykkidou et al., 2009), oxygen scavenger packed cobia (Remya et al 2017). The dominance of LAB was also observed in vacuum packed trout (Lyhs et al., 2001; Knochel, 1983), cold fresh fish fillets (Stamatis & Arkoudelos, 2007). The dominance of Lactic acid bacteria in the present study could be due to the reduced oxygen content inside the packs and also by the action of CO_2. LAB being facultatively anaerobic group, is tolerant to CO_2 and therefore may inhibit growth of other bacteria because of the formation of lacic acid formation and bacteriocins (Dixon & Kell, 1989). Lactic acid bacteria produce organic acids and ethanol as typical fermentation products (Atrea et al., 2009). This may contribute to their selective growth with minor component of gram negative bacteria during spoilage of seafood (Dufresne et al., 2000). Microorganisms like lactic acid bacteria can be stimulated by the action of CO_2. (Coma, 2008).

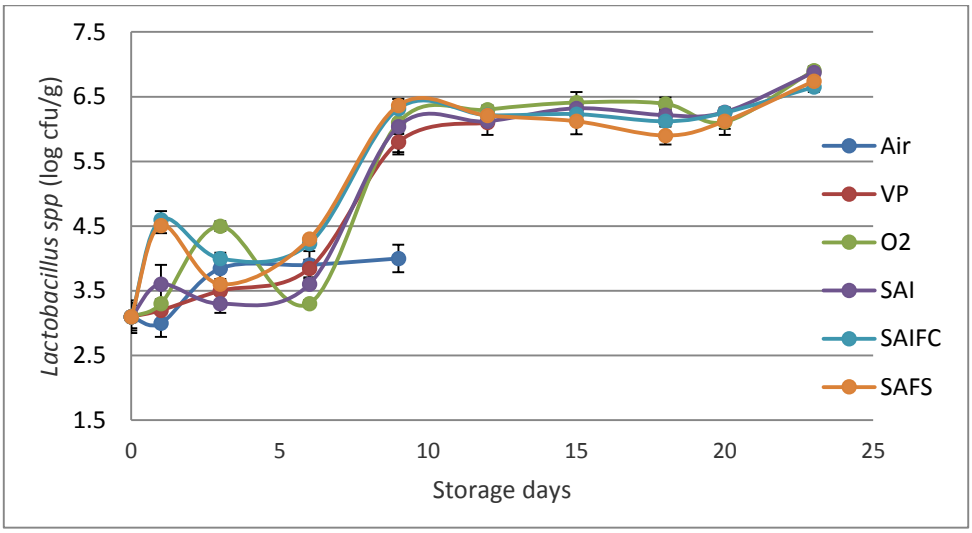


Fig. 8.15. Changes in *Lactobacillus spp.* count of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

8.3.18 Changes in indicator bacteria

The initial *Faecal streptococci* count in yellowfin tuna was 3.77 log cfu per g. At the time of sensory rejection, control air packed samples showed a significantly higher (P<0.05) value (6.1 log cfu/g) compared to the commercial active oxygen scavenger packed yellowfin tuna samples (4.9 log cfu/g). Samples stored under vacuum packs, developed sachets SAI, SAIFC and SAFS showed a count of 5.5, 4.5, 4.1 and 4.3 log cfu/g respectively at the end of sensory rejection. A similar result was also observed for vacuum packed black pomfret and pearl spot (Manju, 2005) seer fish steaks (Mohan et al 2010).

E coli was not observed on the initial day of storage. At the end of storage, these organisms were not detected in any of the samples indicating the inability of *E coli* to grow and survive during chilled storage.

Staphylococus aureus was not detected in yellowfin tuna at any stage of storage. The *E. coli* and *S. aureus* levels were with in the limits prescribed for fresh fish (ICMSF 1986) and it indicates the good microbial quality of product.

8.3.19 *Clostridium botulinum* toxin production

In the present study, *Clostridium botulinum* toxin was not detected in any of samples throughout the storage period. It may be attributed to the good quality of raw materual and maintenance of storage temperature without any temperature abuse. These results are in agreement with the results reported for vacuum packed pearl spot (Manju et al., 2007a), commercially important fish species packed under MAP (Yesudhason 2007), active packed seer fish (Mohan et al., 2010). There is an increasing concern about the growth of psychotrophic pathogens under vacuum packed and modified atmosphere packed food products (Coma 2008).

8.3.20 Changes in K value

K value is widely used as freshness indicator for fish (Massa et al., 2005). The mean initial total molar concentration of nucleotide breakdown products was 11.8 µmol/g, which was higher than the vaue (7.8 µmol/g) reported for cazon fish (Ocaño-Higuera et al., 2009), 8µmol/g for black skip jack muscle (Mazorra-Manzano et al., 2000). Murata & Sakaguchi (1986) observed 9.3 µmol/g for yellow tail muscle. In the present study, the initial ATP concentration was 0.062 µmol/g. According to Haard (1992b), ATP degrades within the first 24 hours of post mortem. An initial ATP concentration of 0.1 µmol/g was observed for ray fish (Ocaño-Higuera, 2011), 0.15 µmol/g for cazon fish (Ocaño-Higuera et al., 2009) 0.1 µmol/g for rainbow trout (Özogul & Özogul, 2002c), 0.18 µmol/g for yellow grouper

(Li et al., 2011), 0.08 for adductor muscle of lions paw scallop (Pacheco-Aguilar et al., 2008). The initial IMP value was 7.4µmol/g which was found be higher than as observed value of 6.35µmol/g for crazon (Ocaño -Higuera et al., 2009) and lower than observed for cultured sea bream (over 10 µmol/g) by Zaragoza et al. (2013). The higher level of IMP in the present study indicates the degradation of ATP. In the present study, a decrease with time was observed for the IMP content during storage period. A similar result was also observed for farm raised sea bass during storage in melting ice (Kyrana & Lougovois, 2002). IMP is considered as flavor enhancer of muscle foods, especially the umami flavor (Kawai et al., 2002) and it provides sweet creamy flavor of fresh fish (Huss, 1995; Aubourg et al., 2007a). The disappearance of IMP has been correlated with the loss of freshness and flavor in some fish species (Howgate, 2005).

In the present study, hypoxanthin was not observed during the initial day of storage. However, the inosine and hypoxanthin level increased significantly during storage period. Post mortem hypoxanthin formation in fish indicates the autolytic and microbial spoilage (Woyewoda et al., 1986) and correlates with loss of sensory quality (Gimenez et al., 2000; Greene & Bernatt-Byrne, 1990). According to Howgate (2005), AMP and IMP are responsible for fresh sweet taste in fish muscle and inosine and hypoxanthin are responsible for the bitter taste during spoilage. In this study, Hx accumulation in fish muscle started in air packed samples from third day reflects initial phase of autolytic deterioration. In this study, the Hx accumulation could be used as freshness indicator due to the correlation of its formation regarding the storage time. The increase of K value during storage is due to the decrease of IMP and its degradation to HxR and Hx.

The concentration of ATP and its degradation products are measured to calculate K vale, a freshness indicator. K value is defined as the ratio of non phosphorylated ATP breakdown products to the total ATP breakdown products (Ehira & Uchiyama, 1987) and it has been used as a frehness indicator in many species. The other freshness indicators Ki, H, G, P and Fr values of yellow fin tuna during chilled storage was also measured in the present study. There was an increasing trend observed in all these parameters during storage (Fig. 8.16.a,b, c, d, e) except for Fr value (Fig.8.16.f). Fig 8.16.a shows a significant increase in K value with storage time in yellowfin tuna during chilled storage. Saito et al. (1959) described fishery products with k value less than 20% as very fresh, less than 50% as moderately fresh and greater than 70% as not fresh. Based on this, the yellowfin tuna meat in the present study was considered as moderately fresh up to 9 day in the case of control air packs and the samples packed with vacuum was fresh up to 10 days. Samples packed with oxygen scavenger, SAI, SAIFC and SAFS were moderately fresh up to 12, 15, 15 and 12 days respectively. The observed K value results were supporting the microbial results in the present study. Tukey's studentized range test showed that all the treatments were significantly ($p<0.05$) different from control air packed samples. The k value of samples packed in active packs showed a significantly lower ($p<0.05$) K value than control air and vacuum packed samples. Tukeys grouping for marginal mean also showed that all treatments were significantly different from control air and vacuum packes samples. This could be explained by the preservative action of carbon dioxide and the absence of oxygen in the packs. A similar result was also reported by Mohan et al. (2010) for seer fish steaks packed in active packs during chilled storage. No off odour and off flavor was detectable in active packed samples until the end of storage. Ehira & Uchiyama (1987) also observed that no spoilage

odour in fish kept at 0^0C until 17^{th} day of storage. K value increased in all samples during storage period suggesting that it can be used as a useful index in studying the freshness of active packed yellowfin tuna during chilled storage.

According to Okuma et al. (1992) usually ATP, ADP and AMP degrade within 24 hours after death and therefore he proposed another index for freshness. Ki value is defined as Ki = (HxR+Hx/IMP+HxR+Hx) X 100. The Ki value observed in the present study is given in fig 8.16.b. Tukey's studentized range test showed that all treatments were significantly different from control air packed samples. Tukey's grouping for marginal mean showed that treatment SAIFC and SAI were homogenous in nature, but significantly different from other treatments. The graph shows a higher Ki value than K value in the present study. The observed maximum Ki values were 89.09%, 94.9%, 92.4%, 84.8%, 82.8%, 84.4% at the end of storage period for control air, vacuum, oxygen scavenger packs and developed dual action sachets with SAI, SAIFC and SAFS respectively. Ki value has been demonstrated as a good freshness index for a large number of species (Ehira & Uchiyama, 1987) and has been widely used as a freshness index (Barat et al., 2008). Özogul et al. (2000) observed an increase in Ki value over chilled storage and reached 90%, 85% and 84% on the day of marginal acceptability during chilled storage of herring packed with air, vacuum and modified atmosphere respectively. Zaragoza et al. (2013) observed an initial Ki value of 8% in seabream. According to Lougovois et al. (2003), very fresh seabream will have Ki value lower than 10%, while fish at the end of shelf life will have a Ki value of 33-35%. Huynh et al. (1992) observed a correlation between sensory fish freshness and the Ki value for sockeye salmon and Pacific herring stored in ice. Luong et al. (1992) observed that

Atlantic cod and Pacific cod rapidly accumulate HxR and thus Ki value is not a suitable quality index for them.

H value rose slower than other values in all treatments during chilled storage. Tukey's studentized range test for H value showed that marginal mean of control air packed samples and vacuum packed samples were homogenous in nature. The samples packed with SAIFC and SAI were homogenous in nature. The samples packed with SAI and SAFS were also homogenous in nature, however SAFS and SAIFC were significantly different from each other. The observed maximum value of H in the present study was 61.9 %, 62.2%, 51.03%, 26.02%, 29.1%, and 31.8% for control air, vacuum, oxygen scavenger packs and developed dual action sachets SAI, SAIFC and SAFS respectively at the end of storage period. Özogul et al. (2004) observed an initial H value of 6% for sardine and a final H value of 62%, 74% and 88% for sardine packed in air, vacuum and MAP. A similar result was also observed in white grouper during chilled storage (Özogul et al., 2008). In the present study, H value formed slowly up to 10 days in the case of active packed samples. The control air packed sample showed a steap rise in H value on 10^{th} day of storage. The sudden increase of H value may be due to the quick decomposition of IMP. Luong et al. (1992) found that H value of pacific cod increased quite steadly to be much superior to the Ki value.

The control air packed samples showed a G value of 205.9 and the vacuum packed samples showed a G value of 235.1 at the end of storage period. Tukey's studentized range test showed that control air and vacuum packed samples were homogenous in nature. Tukeys grouping for marginal mean showed no significant ($p<0.05$) difference between the samples packed with developed sachets. The developed sachets showed significant difference

between commercial oxygen scavenger packs, vacuum and control air packed samples. The active packed samples showed significantly lower ($p<0.05$) G value at the end of storage period having a value of 113.6, 114.5 and 122.5 for oxygen scavenger packs, developed dual action sachets SAI, SAIFC and SAFS. This shows that oxygen scavenger and developed sachets used in the present study were more effective in extending the freshness of samples compared to control air and vacuum packs. G value showed a steady increase on 12^{th} day of storage in control air packs due to the increase in Hx content in samples. A similar result was also observed for farmed turbot (Aubourg et al 2005b), sardine (Özogul et al., 2004), seer fish (Mohan et al., 2009a).

The initial P and Fr values were 23.4% and 76% respectively in the present study. Tukey's studentized range test showed that P value of all treatments were significantly ($p<0.05$) different from control air packed samples. Tukey's grouping for marginal mean showed that P value of SAIFC and SAI were homogenous in nature. P value increased linearly in all samples during storage and Fr value decreased in all samples with time. At the end of storage study, the Fr value reached to 10.9 for control air packed samples. Tukey's studentized range test showed that the Fr value of all treatments were significantly ($p<0.05$) different from control air packed samples. The Fr value for vacuum, oxygen scavenger packs and developed dual action sachets with SAI, SAIFC and SAFS reached a value of 5.07, 7.5, 15.1, 17.14 and 15.5 respectively. In the present study, K, Ki, P, H, G and Fr values provided a useful indicator for assessing freshness of yellowfin tuna during chilled storage.

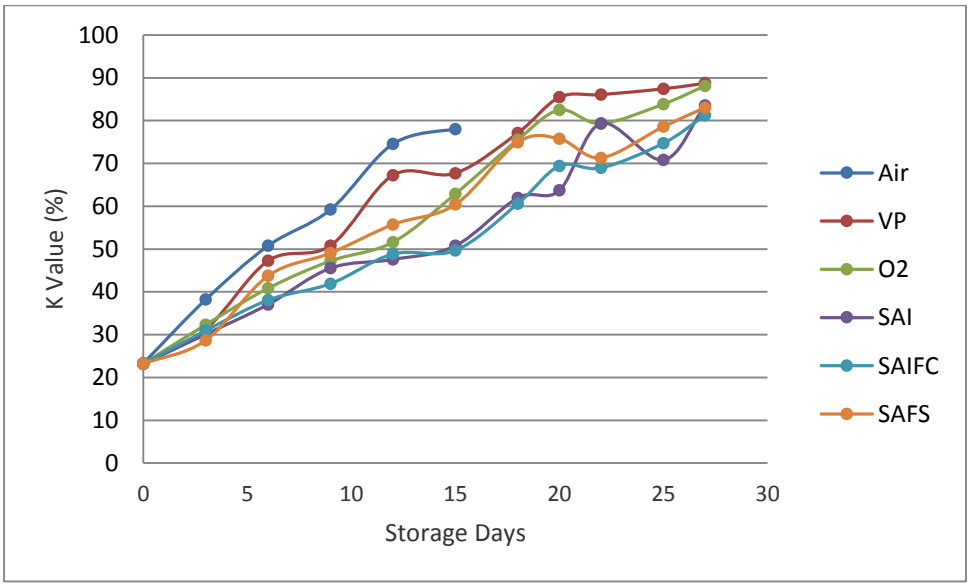


Fig. 8.16.a. Changes in K value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

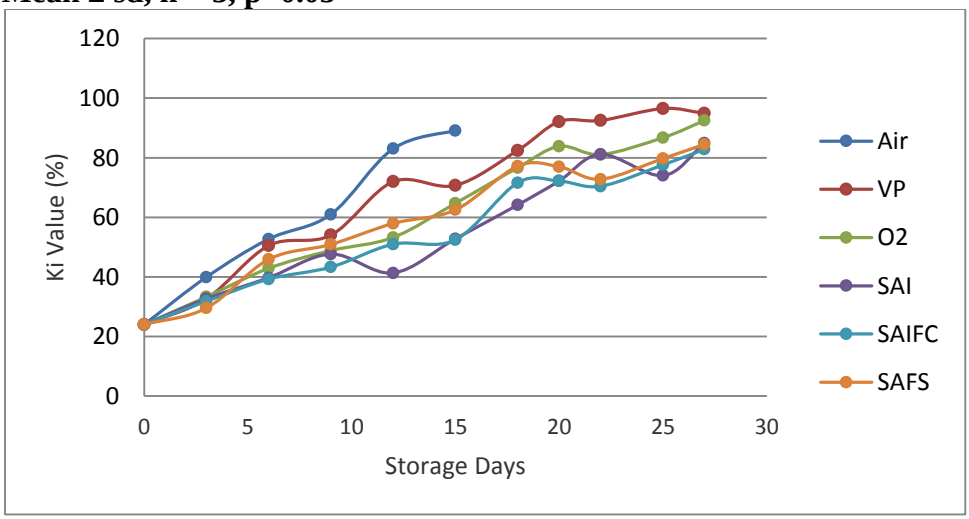


Fig. 8.16.b. Changes in Ki value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

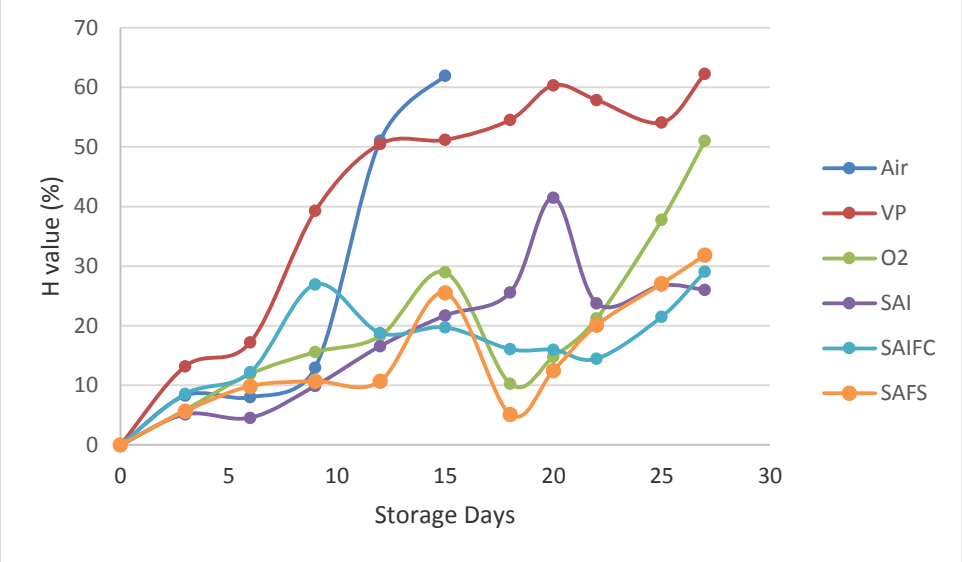


Fig. 8.16.c. Changes in H value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

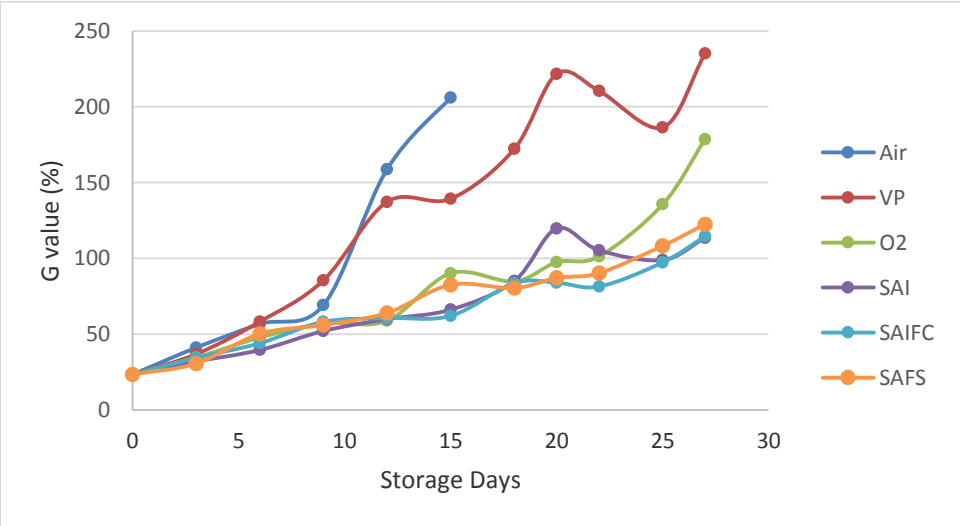


Fig. 8.16.d. Changes in G value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

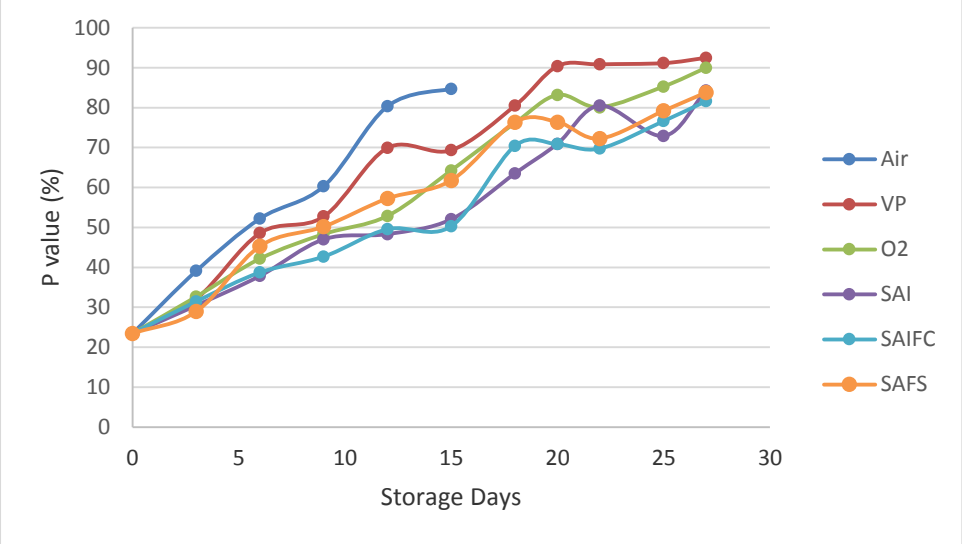


Fig. 8.16.e. Changes in P value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

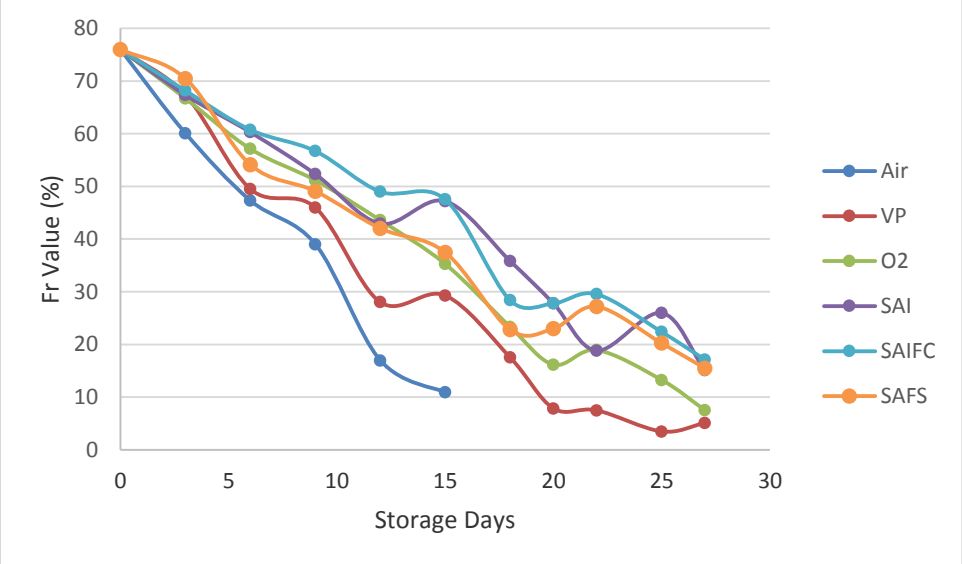


Fig. 8.16.f. Changes in Fr value of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

8.3.21 Changes in Biogenic amines

Fish is a rich source of high quality proteins, which represents a risk in the decomposition processes. Disintegration of proteins leads to the production of peptides and aminoacids, which further disintegrates to form biogenic amines and other compounds (Zarei et al., 2011). Histamine is produced in raw fish by the action of bacterial histidine decarboxylase. It has been considered as the contributory toxin of scombroid fish poisoning (Önal, 2007) and FDA identified histamine as a major chemical hazard in seadood products (FDA, 1998). A maximum histamine content of 50 mg/kg (50ppm) has been established by FDA (2001) and 200 mg/kg by EC (1991) for acceptance of tuna and other fishes belonging to scombridae and scomberesocidae families. According to Parente et al. (2001) histamine intake range within 8-40 mg, 40-100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning respectively. Histamine alone appears not to be the sole factor in causing toxicity since toxicity was observed even at low levels of histanmine concentration. Biogenic amines such as putrescine, cadaverine, spermine, spermidine in fish tissue may potentiate the toxic effect of histamine by inhibiting the intestinal histamine metabolizing enzymes diamine oxidase and histamine N methyltransferase (Stratton et al., 1991). Putrescine and spermidine have no adverse effect on health, but it may react with nitrite to form carcinogenic nitrosamines.

The concentration of histamine, putrescine, cadaverine, spermine, spermidine, tyramine in yellowfin tuna during chilled storage is given in Fig. 8.17.a, b, c, d, e, f respectively. In the present study, histamine was not detectable during the initial day of storage. A similar result was also observed for yellowfin tuna (Du et al., 2002), skipjack tuna (Frank et al., 1981), red drum (Cai et al., 2015), tuna (Veciana-Nogués et al., 1997). According to

Auerswald et al., (2006), histamine levels in freshly caught fish are generally low, usually below 0.1mg/100g. Tukey's studentized range test for histamine showed that all treatments were significantly ($p<0.05$) different from control air packed samples. Tukey's grouping for marginal mean showed no significant difference between the samples packed with vacuum, commercial oxygen scavenger and developed sachets SAI, SAIFC and SAFS. During storage the histamine concentration increased in all packs, however the histamine formation was significantly higher ($p<0.05$) in control air packed samples compared to active packed samples. This may be due to the combined protective action of CO_2 and reduced oxygen concentration inside the packages. At the end of storage, the histamine content in control air packed samples reached 68.4 mg/kg wheras the samples packed in active packs never reached this value during storage. The results indicate that the formation of histamine was greately effected by the use of developed dual action sachets and commercial oxygen scavenger packs. The results are in agreement with the results reported by Mohan et al. (2009) on the effect of commercial oxygen scavengers in biogenic amine formation in seer fish under chilled storage. Histamine formation in big eye tuna and skipjack tuna during storage at 4^0C, 10^0C and 22^0C was studied by Silva et al. (1998). Histamine level reached 500ppm (50 mg/100g) on first and second day of storage at 22^0C for skipjack and big eye tuna respectively. The histamine formation delayed during refrigerated storage at 4^0C and 10^0C. Notable amount of histamine could be detected after 3 days and 6 days of storage at 4^0C and 10^0C respectively. Du et al. (2002) observed that in yellowfin tuna stored at 0^0C did not reach the upper acceptability limit of 50ppm even after 9 days of storage. Yesudhason et al. (2013) studied the levels of histamine in commercially important fish species of Oman. He observed that among

detected samples, the level of histamine varied from 1-229mg/kg with an overall mean value of 2.6 mg/kg.

In the present study, amines were not detected on the first three days of storage except for putrescine. Cadaverine and putrescine due to their ability to potentiate histamine toxicity is very important especially in scombroid fishes (Alak et al., 2010). FDA (1996) recommended putresciene, and cadaverine for judgement of fish freshness. In the present study, large changes in the contents of putresciene and cadaverine was observed throughout the chilled storage period of yellowfin tuna. Tukey's studentized range test for putrescine showed that all treatments were significantly ($p<0.05$) different from control air packed samples. Tukey's grouping for marginal mean showed that samples packed with SAIFC and SAI were homogenous in nature. The concentration of putrescine increased in all packs and reached a maximum value of 5.54 mg/kg in the case of control air packs at the end of storage. The development of putrescine in developed sachets and commercial oxygen scavenger packs were significantly slower ($p<0.05$) than the control air and vacuum packed samples with respect to storage time. This could be attributed to the effect of carbon dioxide and the reduced oxygen content inside the packs thereby inhibiting the microorganisms responsible for the formation of biogenic amines. The results are in agreement with the tuna packed in air vacuum and modified atmosphere packs (Ruíz-Capillas and Moral 2005), seer fish steeks packed under reduced oxygen concentrations (Mohan et al 2009), herring under modified atmosphere (Özogul et al., 2002a, 2002b). Putrescine was the second highest biogenic amine in barramundi fillets. Cantony et al. (1993) observed 250ppm of cadaverine in spoiled samples of smoked salmon. Bakar et al. (2010) observed putrescine (362.4), cadaverine (158.7) and tryptamine (30.5) mg/kg

as the dominant biogenic amines at the end of chilled storage in barramundi. Valle et al. (1996) observed that putrescine and cadaverine content reached a value of 10.1 and 23mg/kg in herring (*Clupea herengus*) at the end of storage period at 0^0C.

In the present study, cadaverine was not observed in the initial day of storage. The value then increased up to 30.21 mg/kg on 18^{th} day of storage in the case of control air and 26.34, 25.8, 20.21 and 24.89 mg/kg on 29^{th} day of storage in the case of samples packed with oxygen scavenger, developed sachets SAI, SAIFC and SAFS respectively. Tukey's studentized range test for cadaverine showed that all treatments were significantly ($p<0.05$) different from control air packed samples. The cadaverine formation was significantly lower ($p<0.05$) in samples packed in active packs. It may be due to the protective effect of active packaging against cadaverine formation. According to Yassoralipour et al. (2012), Cadaverine was the major amine found in baramundi fillets stored under chilled conditions. He observed 279.0, 295.6, 316.7, 339.7 and 538.7 mg/kg of cadaverine at the end of 20 days of different storage conditions.

In the present study initially spermidine and spermine was not observed. The spermidine value fluctuated during the storage period in the case of active packed samples. Tukey's studentized range test for spermidine showed that all treatments were significantly ($p<0.05$) different from control air packed samples. Tukey's grouping for marginal mean showed that treatment SAI and SAFS were homogenous in nature, but significantly different from other treatments. The spermine value slowly increased in all samples during storage and reached 20.6 mg/kg on 18^{th} day of storage in the case of control air packs. Tukey's studentized range test for spermine showed that all treatments were significantly ($p<0.05$) different from control air

packed samples. Tukey's grouping for marginal mean showed that vacuum packed samples and commercial oxygen scavenger packs were homogenous in nature. Samples packed with SAFS and SAIFC were also homogenous in nature. The vacuum and oxygen scavenger packed samples reached 20.2mg/kg spermine on 24th day of storage. The developed sachets never reached this value till the end of storage.

Maximum tyramine level recommended for food is 100-800mg/kg (Yassoralipour et al., 2012). Tyramine has vasoactive and psychoactive properties along with its adverse reactions involving monoamine oxidase inhibitor drugs. Tukey's studentized range test for spermidine showed that all treatments were significantly ($p<0.05$) different from control air packed samples. In the present study, tyramine was observed only after 7th day of storage in the case of samples packed in vacuum, oxygen scavenger and other developed active packing systems. Tyramine concentration of 4.5 mg/kg was observed in samples packed in air on 18th day of storage. Wheras a level of 2.2, 2.1,2.4 and 3.2 mg/kg was observed for samples packed with oxygen scavenger, developed sachets SAI, SAIFC and SAFS indicating the effectiveness of oxygen scavenger and dual action sachets in inhibiting the tyramine formation. Similar results were reported for seer fish (Mohan et al., 2009), carp (Krizek et al., 2004) and sardine (Özogul et al., 2004) stored under different conditions. In the present study the total content of putresciene, cadaverine and histamine of yellow fin tuna packed in control air packs on 18th day of storage was 104.1mg/kg. Total content of puteresciene, cadaverine, and histamine can be used as an indicator for tuna spoilage and scombroid fish poisoning (Rosier & Van Peteghem, 1988).

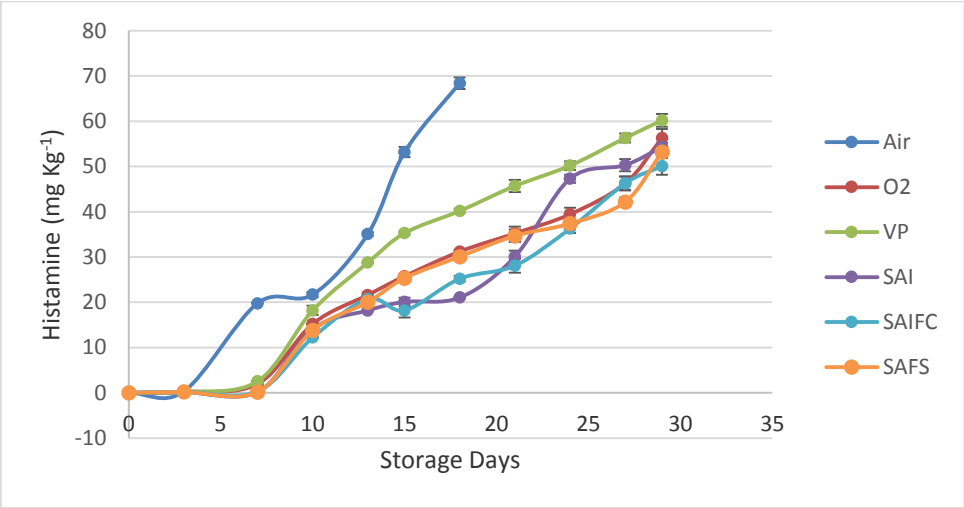


Fig. 8.17.a. Changes in histamine content of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

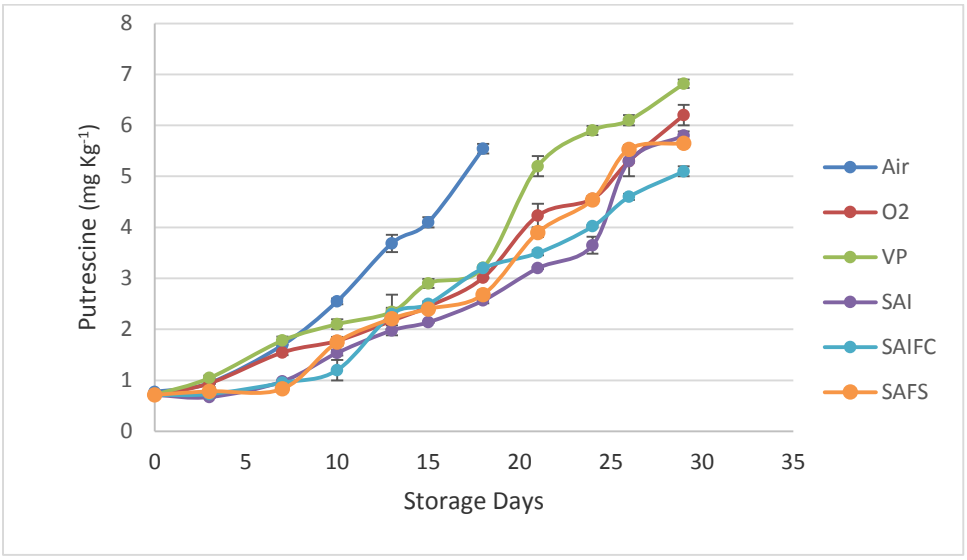


Fig. 8.17.b. Changes in putrescine content of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

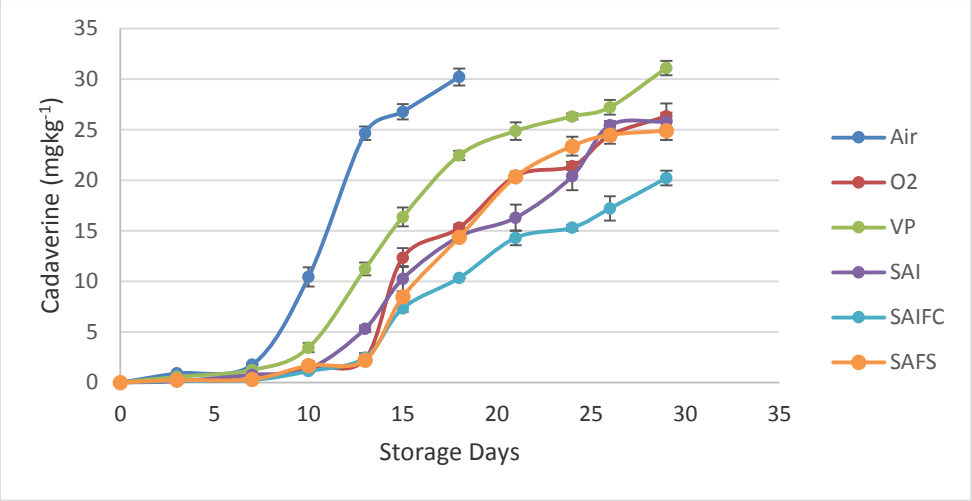


Fig. 8.17.c. Changes in cadaverine content of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

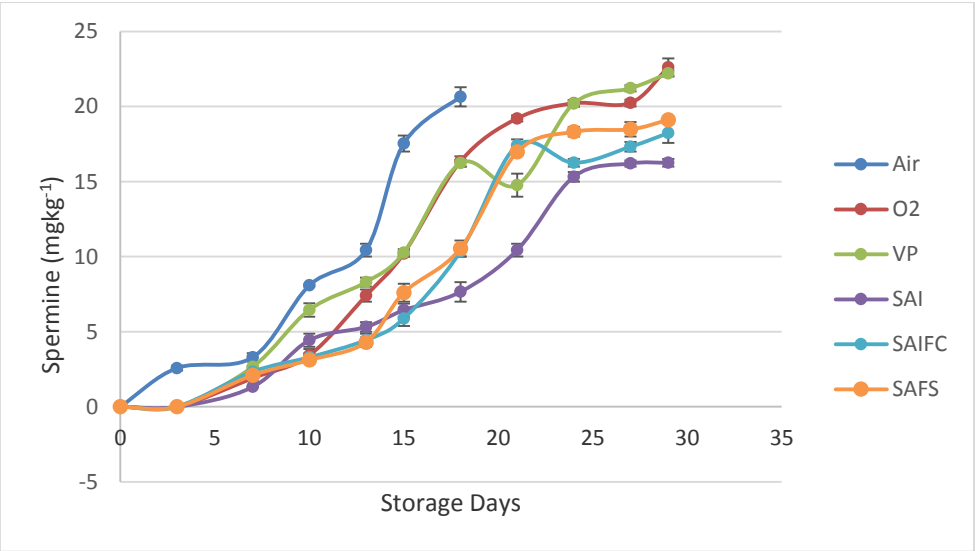


Fig. 8.17.d. Changes in spermine content of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

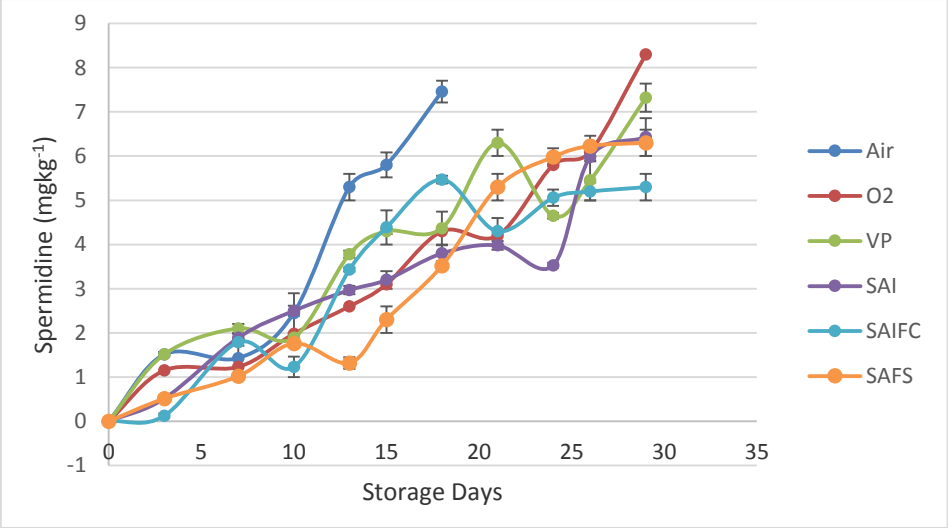


Fig. 8.17.e. Changes in spermidine content of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

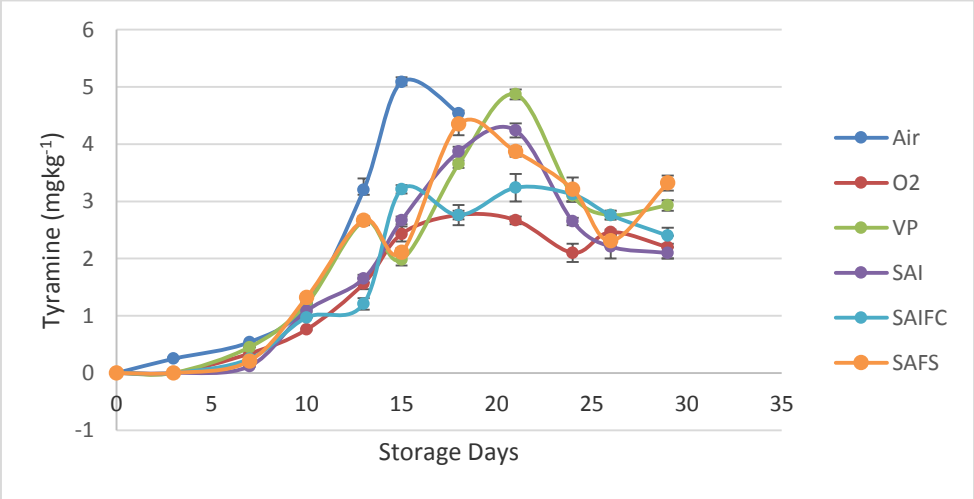


Fig. 8.17.f. Changes in tyramine content of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

8.3.22 Quality index and biogenic amine index

Fig. 8.17.g and Table 8.2 shows the biogenic amine index and quality index of yellowfin tuna stored under different atmospheric conditions respectively. There was an increase in two indices with storage time indicating that it can be used as an indicator for determining the spoilage of yellowfin tuna. Mieltz & Karmas (1977) proposed a value of 10 as the limit of acceptability for the quality index (QI). But in the present study, the samples did not reach a value of 10 during the study period. That may be due to the higher concentration of spermine and spermidine observed in the present study. The biogenic amine index (BAI) proposed by Veciana-Nogués et al. (1997) correlated well with sensory acceptability and microbial count of yellowfin tuna packed under various atmosphere conditions. Yellowfin tuna chunks packed in air and vacuum exhibited more biogenic amine values than in active packed samples. Özogul & Özogul (2006) observed a twice higher biogenic amine index value than quality index in sardine stored under refrigerated controlled temperature of 4^0C.

Table. 8.2. Quality index (biogenic amine) score of yellowfin tuna packed under different atmosphere conditions during chilled storage

Quality index	Storage days										
	0	3	7	10	13	15	18	21	24	27	29
air	0.770	0.431	4.061	3.009	3.790	3.455	3.579				
O_2	0.715	0.629	0.924	2.896	2.374	2.836	2.290	2.455	2.422	2.783	2.786
VP	0.715	0.712	0.967	2.545	3.237	3.509	3.050	3.434	3.188	3.240	3.215
SAI	0.715	0.652	0.457	2.143	2.747	3.056	3.056	3.219	3.594	3.496	3.628
SAIFC	0.715	0.959	0.279	2.635	2.873	2.492	2.304	2.022	2.493	2.895	3.074
SAFS	0.715	0.809	0.326	2.939	3.696	3.323	3.131	2.533	2.581	2.858	3.174

(AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets)

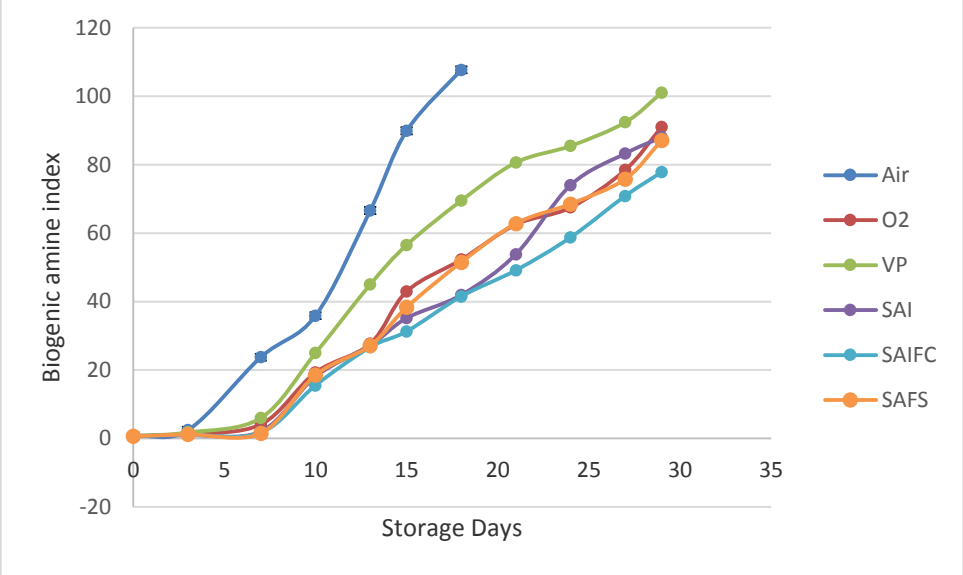


Fig. 8.17.g. Biogenic amine index of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets)

8.3.23 Changes in Texture

Texture is one of the key quality attribute to measure the quality and freshness of food (Chen & Opara, 2013) since it affects the mechanical processing of fillets in seafood industry. Food texture is defined as all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactical and where appropriate visual and auditory receptors (Lawless & Heymann, 1998). The texture perceived in the mouth depends on the mechanical behavior of food and it determines the dynamics of breakdown during eating food (Foegeding et al., 2010). Texture loss during storage and spoilage has been studied by several authors and according to De Vido et al. (2001) tough texture and high drip loss is associated with low muscle pH. Sato et al. (1991) suggest the involvement of several enzymes in texture deterioration durning storage. Texture profile

analysis is based on the imitation of mastication or chewing process with a double compression cycle. The hardness changes during chilled storage of yellowfin tuna in the present study is given in Fig. 8.18.a and b. There was a decrease in both hardness 1 and 2 in all samples during storage. The Tukey's Studentized range test for hardness 1 showed that all the treatments were significantly ($p<0.05$) different from control air packed samples. The marginal mean for treatments showed that among the treatments, commercial oxygen scavenger packs and SAFS were homogenous in natire. The combinations SAIFC and SAFS were also homogenous in nature. But SAIFC was significantly different from commercial oxygen scavenger packs. The Tukey's Studentized range test for hardness 2 showed that all the treatments were significantly ($p<0.05$) different from control air packed samples. The samples packed with developed sachets showed relatively lesser softening of muscle during storage. The decrease of hardness in the present study can be attributed to the weakening of connective tissue of yellowfin tuna during storage. Gallart-Jornet (2007), Erikson et al. (2011) observed that hardness of salmon fillets did not change during super chilled storage. The results in the present study are in agreement with those of Azam et al. (1989) who observed significant softening of rainbow trout during storage in ice. Lee & Toledo (1984) observed softening of mullet during chilled storage. Manju et al. (2007a,b) observed softening of pearl spot during chilled storage.

The springiness of samples was tested to simulate finger feel of raw sample. Springiness is the elastic or recovering property of the fish muscle during compression. Changes in springiness of yellowfin tuna during storage is given in Table 8.3. The Tukey's studentized range test for springiness showed that all the treatments were significantly ($p<0.05$) different from control air packed samples. The springiness also showed a

decreasing trend in all samples during storage. The marginal mean for treatment showed that vacuum packed sample and SAFS were homogenous in nature. The values in the present study indicated that elasticity of fish muscle reduced during storage.

Cohesiveness is the extent to which the sample could be deformed prior to rupture. The Tukey's studentized range test for cohesiveness (Table. 8.4) showed that all the treatments were significantly ($p<0.05$) different from control air packed samples. The marginal mean for treatment showed that samples packed in vacuum, developed sachets SAI, SAIFC and SAFS were homogenous in nature. There was no significant difference observed between the samples packed with developed sachets. Chewiness represents the work done (Chen & Opara 2013). The Tukey's studentized range test for chewiness (Table 8.5) showed that all the treatments were significantly ($p<0.05$) different from control air packed samples. The samples packed with commercial oxygen scavenger and SAIFC were homogenous in nature. Chewiness showed a decreasing trend during storage in all samples. Decrese in chewiness indicates that the fish muscle become soft during storage. Stiffness is the resistance of a viscoelastic body from the deflection, when the force is applied (Mohsein, 1970). The Tukey's studentized range test for stiffness (Table 8.6) showed that there was no significant difference ($p <0.05$) found between the samples packed with control air, SAI and SAFS. Samples packed with developed sachets were homogenous in nature. According to Ayala et al. (2010), all parameters except springiness decreased with post mortem storage of seabream. Fagan et al. (2004) observed no effect on the springiness of whiting, mackerel and salmon portions during storage at modified atmosphere conditions. Li et al. (2011) observed that the average values for hardness, gumminess and

chewiness of yellow grouper showed significant variations on different sampling days and correlated significantly with storage time. Fish muscle texture depends on the intrinsic factors like muscle fibre density, fat and collagen content of fish muscle. Autolytic and microbial spoilage after death makes the fish muscle softer and less elastic (Olafsdottir et al., 2004). The decrese in textural properties may be associated with the degradation, water loss, deterioration, and oxidation (Delbarre - Ladrat et al., 2006). However in developed dual action sachets, which emits CO_2 and scavenges O_2 in the packs, extended the deterioration period considerably compared to oxygen scavenger packs, vacuum and air packs.

Table 8.3. Changes in the Springiness of yellowfin tuna chunks during chilled storage at various packaging treatments

Days	Treatment						Mean Tukey's test for day
	Air	Vacuum	O_2	SAI	SAIFC	SAFS	
0	7.11	7.11	7.11	7.11	7.11	7.11	**7.1^{a}**
5	2.04	3.44	3.18	2.85	2.95	3.04	**2.9^{e}**
10	4.36	2.89	2.65	3.2	3.86	2.45	**3.2^{d}**
15	2.19	2.9	4.67	4.47	4.25	3.8	**3.72^{c}**
20	3.69	4.2	3.8	4.75	4.22	3.5	**4.04^{b}**
25	2.3	2.91	2.26	2.59	2.8	3.5	**2.7^{f}**
30	2.33	4.17	2.16	5.57	3.73	4.38	**3.7^{c}**
Marginal mean for treatment	**3.4^{e}**	**3.9^{c}**	**3.7^{d}**	**4.36^{a}**	**4.13^{b}**	**3.9^{c}**	

Tukey's Studentized Range (HSD) test result for Springiness, Means with the same letter are not significantly different. (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS=developed dual action sachets) n=3, $p<0.05$

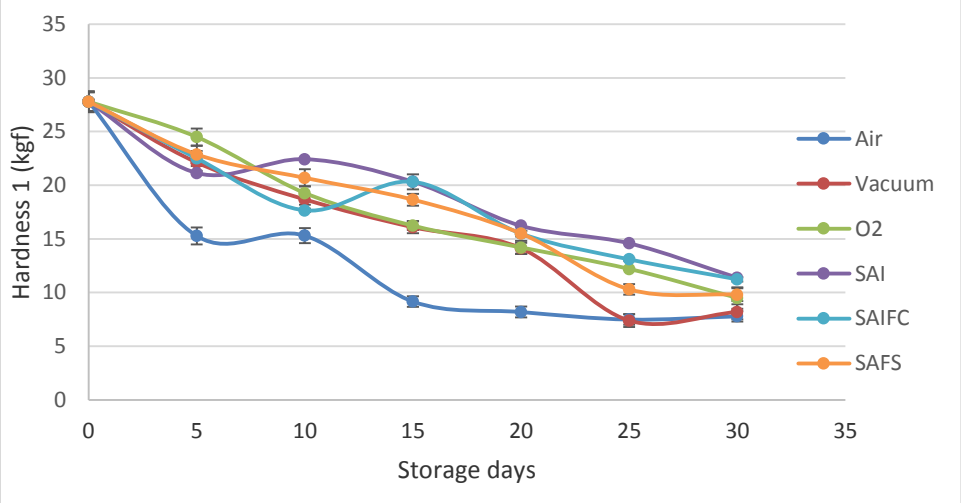


Fig. 8.18.a: Changes in hardness 1 of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

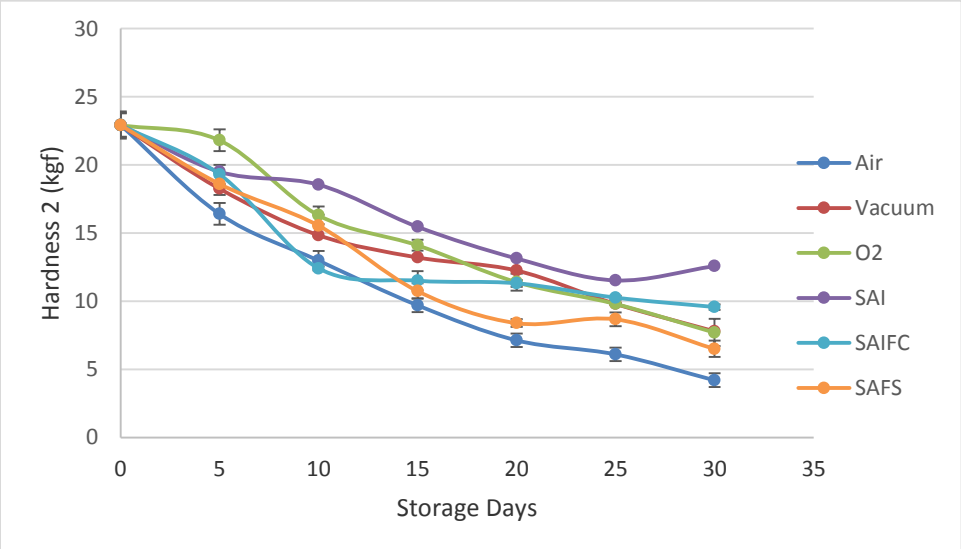


Fig.8.18.b : Changes in hardness 2 of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS = developed dual action sachets) *Mean ± sd, n = 3, p<0.05

Table 8.4. Changes in the Cohesiveness of yellowfin tuna chunks during chilled storage at various packaging treatments

Days	Treatment						Mean Tukey's test for day
	Air	Vacuum	O2	SAI	SAIFC	SAFS	
0	0.44	0.44	0.44	0.44	0.44	0.44	**0.44^{a}**
5	0.51	0.31	0.32	0.22	0.25	.26	**0.31^{c}**
10	0.77	0.27	0.19	0.36	0.36	0.25	**0.37^{bc}**
15	0.36	0.29	0.27	0.28	0.29	0.38	**0.31^{c}**
20	0.28	0.41	0.44	0.39	0.43	0.38	**0.39^{ab}**
25	0.34	0.52	0.21	0.31	0.37	0.34	**0.35^{bc}**
30	0.41	0.39	0.36	0.39	0.35	0.48	**0.39^{ab}**
Marginal mean for treatment	**0.45^{a}**	**0.38^{b}**	**0.32^{c}**	**0.34^{bc}**	**0.35^{bc}**	**0.36^{bc}**	

Tukey's Studentized Range (HSD) test result for Cohessiveness, Means with the same letter are not significantly different. (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS=developed dual action sachets) n=3, $p<0.05$

Table 8.5. Changes in the Chewiness of yellowfin tuna chunks during chilled storage at various packaging treatments

Days	Treatment						Mean Tukey's test for day
	Air	Vacuum	O_2 Scavenger	SAI	SAIFC	SAFS	
0	8.5	8.5	8.5	8.5	8.5	8.5	**8.5^{a}**
5	4.1	9.8	8.8	1.04	2.4	1.49	**4.6^{c}**
10	3.63	2.43	8.79	2.09	1.57	0.41	**3.15^{e}**
15	1.83	1.35	6.2	5.61	5.85	2.67	**3.95^{d}**
20	1.67	1.67	1.75	11.4	10.73	7.58	**5.8^{b}**
25	0.55	0.75	1.32	0.92	1.58	1.18	**1.05^{f}**
30	0.63	0.78	1.37	12.42	5.88	7.17	**4.7^{c}**
Marginal mean for treatment	**3.01^{e}**	**3.63^{d}**	**5.3^{b}**	**6.0^{a}**	**5.23^{b}**	**4.16^{c}**	

Tukey's Studentized Range (HSD) test result for chewiness, Means with the same letter are not significantly different. . (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS=developed dual action sachets) n=3, $p<0.05$

Table 8.6. Changes in the Stiffness of yellowfin tuna chunks during chilled storage at various packaging treatments

Days	Treatment						Mean Tukey's test for day
	Air	Vacuum	O_2	12	14	16	
0	0.58	0.58	0.58	0.58	0.58	0.58	**0.58^{f}**
5	1.14	0.58	1.75	0.44	1.89	0.71	**1.08^{b}**
10	1.07	0.72	0.67	0.95	0.39	0.28	**0.672^{e}**
15	0.68	0.47	1.37	1.33	0.96	0.92	**0.95^{c}**
20	0.68	1.36	1.46	2.05	2.06	2.27	**1.64^{a}**
25	0.95	0.38	1.37	0.77	0.97	0.35	**0.8^{d}**
30	2.83	1.47	1.34	1.38	0.57	2.36	**1.66^{a}**
Marginal mean for treatment	**1.13^{b}**	**0.79^{d}**	**1.2^{a}**	**1.07^{bc}**	**1.06^{c}**	**1.07^{bc}**	

Tukey's Studentized Range (HSD) test result for Stiffness. Means with the same letter are not significantly different. (AIR = control air packed sample, VP = vacuum packed sample, O_2 = samples with commercial oxygen scavenger, SAI, SAIFC & SAFS=developed dual action sachets) n=3, $p<0.05$

8.3.24 Changes in drip loss

Drip loss directly effects the sensory and other physicochemical attributes of fish like waterholding capacity, juciness, flavor, appearance, and texture (He et al., 2014).The increased driploss leads to the loss of water soluble nutrients like proteins, amino acids and vitamins providing a nutritious medium for bacteria (Liu et al., 2013). Tukey's studentized range test for drip loss showed that all treatments were significantly ($p<0.05$) different from control air packed samples. Tukey's grouping for marginal mean showed that among treatments, vacuum pack and SAFS were homogenous. All other treatments were significantly different from each other. In the present study, drip loss increased in all samples during storage period. The changes in drip loss of yellow fin tuna during chilled storage are shown in Fig. 8.19. A rapid increase in drip loss was observed in samples packed in air. A gradual increase in drip loss was observed in samples packed with vacuum and other active packed samples. Drip loss was rapid after 15

days of storage in sampes packed in vacuum and active packs. In the present study, it was observed that samples packed in commercial active oxygen scavengers showed least drip loss at the end of storage compared to the developed dual action sachets. This may be due to the effect of CO_2 in the dual action sachets. According to Masniyom et al. (2002) and Dalgaard et al. (1993) the higher the content of CO_2 in MAP, the higher the drip loss. This may be due to the loss of water holding capacity of muscle protein at lower pH values due to the dissolution of CO_2 in the aqueous phase of fish muscle (Pastoriza et al., 1998; Sivertsvik et al., 2002a). The Drip loss refers to the most loosely bound water in muscle and it is associated with the structure of muscle and muscle cells, denaturation of proteins and rigor stage of muscle (Huff-Lonergan, 2005). Dalgaard et al. (1993) observed 4.7% drip loss in vacuum packed cod fillets at the time of sensory rejection. Liu et al. (2013) observed a higher drip loss for fish fillets stored at -3^0C compared with those stored at 0^0C. Zaragoza et al. (2013) observed slight increase in drip loss in seabream during chilled storage due to the protein denaturation that took place in fish muscle causing softening of tissue and loss of water holding capacity. Fagan et al. (2004) observed no effect on drip values for salmon, mackerel and whiting.

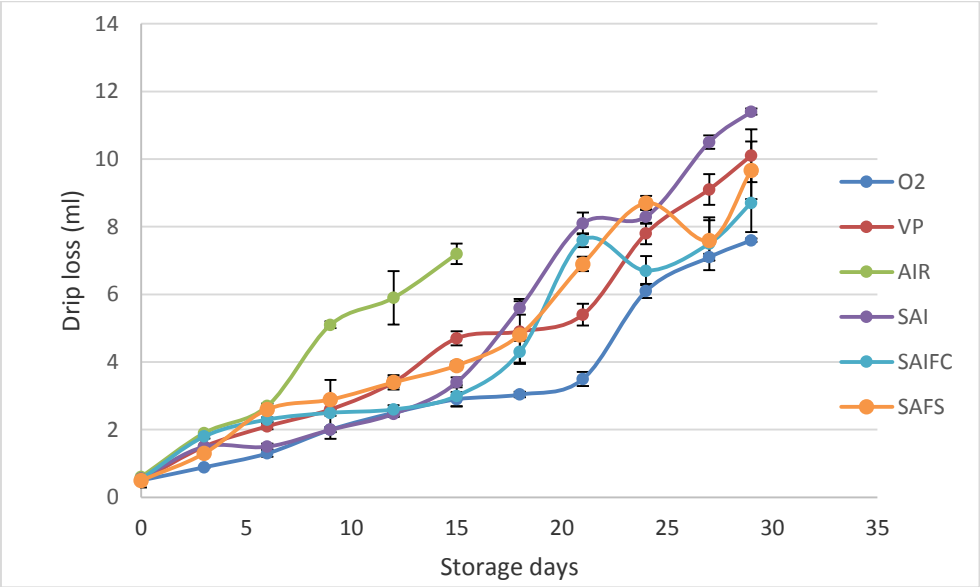


Fig. 8.19. Changes in drip loss of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with active oxygen scavengers, SAI, SAIFC and SAFS = developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.3.25 Changes in Waterholding capacity

Water holding capacity and related liquid loss is an import quality parameter since it affects both profitability and quality of seafood. It influences the appearance of fish muscle before and after cooking. It affects the weight during transportation and storage, drip loss during thawing, weight loss and shrinkage during cooking and tenderness of the meat (Shaviklo et al., 2010). The juiceness of fish muscle and weight loss during cutting and storage depends on the waterholding capacity of fish muscle (Olsson, 2003) and drip loss represents the most loosely bound water (Duun & Rustad, 2008). In the present study, all samples showed a decreasing waterholding

capacity during storage period. Tukey's studentized range test for water holding capacity showed that all treatments were significantly ($p<0.05$) different from control air packed samples. However, Tukey grouping for marginal mean showed that samples packed with commercial oxygen scavenger, SAFS, SAIFC and SAI were homogenous in nature. Treatment SAI and vacuum pack were also homogenous in nature, but vacuum pack were significantly different from other active packed samples. A slight increase in waterholding capacity was observed in all samples after sixth day of storage and afterwords a decresasing trend showed in all samples. There was no significant difference ($P<0.05$) in water holding capacity amoung the samples packed in different atmosphere conditions. The average initial water holding capacity of yellowfin tuna meat was 13.45% (Fig. 8.20).Waterholding capacity is considered as an indictor of fish muscle freshness since its decrese results in texture loss (Hsing-Chen et al., 1990; Sato et al., 1991). Fish muscle becomes tougher by progressive loss of liquid and reduction of water holding capacity. According to Flores & Bermell (1984), decrease in water holding capacity is due to the loss of functionality of of the myofibrillar proteins which is responsible for water retention in fish muscle. Erikson et al. (2011) observed that Atlantic salmon stored in slury ice showed a reduction in WHC over time. Bao et al. (2007) also observed a reduction in WHC during superchilling of Atlantic char. According to Fik et al. (1988), super chilling gradually reduces the WHC with time and drip loss mainly depends on the microstructure of flesh.

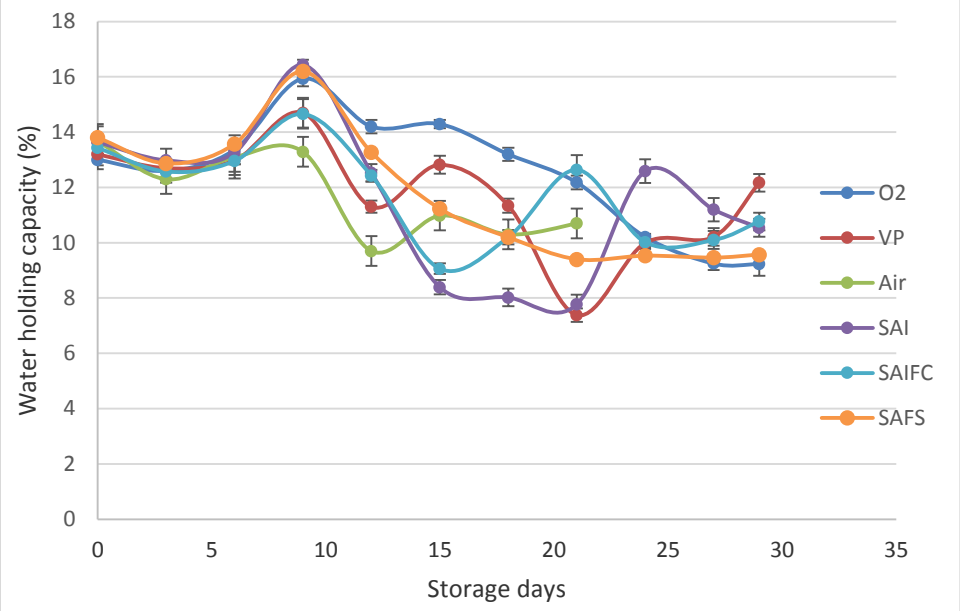


Fig. 8.20. Changes in water holding capacity of yellowfin tuna packed under different atmosphere conditions during chilled storage (AIR = control air packed samples, VP = vacuum packed samples, AP = samples with active oxygen scavengers, SAIFC and SAFS= developed dual action sachets) *Mean ± sd, n = 3, $p<0.05$

8.4 Conclusion

The results of the present study reveals that dual action sachets with carbon dioxide releasing and oxygen scavenging capacity can be used to prolong the shelf life of yellowfin tuna during chilled storage. In the present study acitve oxygen scavengers and carbon dioxide emitters significantly ($p<0.05$) delayed the microbial growth and extended the shelf life of yellowfin tuna. Samples packed with control air reached the upper microbial acceptability limit on 9th day of storage. However the samples packed with vacuum, commercial oxygen scavenger and developed sachets SAI, SAIFC and SAFS crossed the microbial acceptable limit on 12th, 18th, 20th, 22nd and

18th days of storage respectively. This indicates a shelf life of 9-12, 15-18, 18-20, 20-22 and 15-18 days for vacuum, oxygen scavenger and developed sachets SAI, SAIFC and SAFS respectively. *C botulinum* toxin was not observed in any of the samples during storage. The sensory score of samples packed with air and vacuum packs correlate well with the microbial counts during storage. However, the sensory score was not in accordance with the microbial score of all active packed samples. Hence the microbial count limited the shelf life of active packed samples. The freshness indicators like K value, Ki value, G value, H value, P value and Fr value correlated well with the microbial results of active packed samples. The biogenic amine index was also correlated well with sensory acceptability and microbial count of yellowfin tuna packed under various atmosphere conditions. The physicochemical and microbial studies of yellowfin tuna chunks stored under various packaging treatments showed that among the developed chemical combinations, SAIFC exhibited the best active packaging functions to improve the quality and shelf life of yellowfin tuna during chilled storage.

Chapter - 9
SUMMARY AND CONCLUSIONS

Yellowfin tuna being one of the commercially important fish species, was selected as the raw material for the present study. Ethylene vinyl alcohol co-polymer with high barrier properties was used as the packaging material to store yellowfin tuna during chilled storage. High molecular weight high density polyethylene film with very low barrier properties was used as the packaging material for making dual action sachets. Eighteen different chemical combinations were used to develop dual action scavengers with oxygen scavenging and carbon dioxide emitting capacity. The dual action sachets were tried with the principle that when bicarbonate compounds react with acids, carbon dioxide is released along with the formation of other compounds. In the present study, iron powder and ascorbic acid were used for oxygen scavenging, where ascorbic acid accelerates the scavenging action. Sodium bicarbonate was used as a medium for carbon dioxide emission. The developed sachets were tested for the liberation of CO_2 and for the absorption of O_2 quantitatively by using gas analyzer. Developed active packaging sachet containing sodium bicarbonate:ascorbic acid:iron powder (SAI) (2:2:1); sodium bicarbonate:ascorbic acid:iron powder:ferric carbonate (SAIFC) (1:2:1:1); sodium bicarbonate:ascorbic acid:ferrous sulphate (SAFS) (2:2:1) exhibited the best dual action of CO_2 emission along with O_2 scavenging in the package headspace throughout the storage days. For the selection of best dual action sachets, marginal means of treatment and storage days were computed and compared by Tukey's test. A desirability score was

computed and cross checked with the Tukey's test before selecting the treatments using a code in SAS 9.3.

The quality characteristics of yellowfin tuna was analysed and observed that yellowfin tuna protein is well balanced in amino acid composition. Proximate composition of yellowfin tuna showed 73.28% moisture, 1.52% crude fat, 23.18% crude protein and 1.52% ash. The most abundant mineral in yellowfin tuna meat was potassium followed by sodium. The average calcium and iron content in yellowfin tuna meat was 381.80 ppm and 101.86 ppm respectively. The most predominant essential amino acids were lysine and leucine, while tryptophan was the essential amino acid with the lowest concentration. Glutamic acid constituted the highest non-essential amino acid. The percentage ratio of essential amino acid to total amino acid was 48.2%. Yellowfin tuna meat was found to be rich in DHA and EPA (45.14% and 5.51% respectively). Saturated fatty acid, mono unsaturated fatty acid and PUFA contents of raw tuna were 31.19%, 8.23% and 58.79%, respectively. The major SFA were palmitic (C16:0) and stearic (C18:0) acid. The major MUFA were palmitoleic (C16:1) and oleic (C18:1) acid. The n-3/n-6 poly unsaturated fatty acid ratio was 6.78, showing that yellowfin tuna meat is rich in n-3 PUFA. The present study shows that yellowfin tuna meat is a good source of PUFA.

The application of commercial oxygen scavengers on the shelf life extension of yellowfin tuna during chilled storage was compared with control air and vacuum pack. The commercial oxygen scavengers were able to reduce the oxygen concentration inside the packs to below 0.01% within 24 hours and it maintained the reduced oxygen level till the end of storage period. It was observed that oxygen scavenger significantly ($p<0.05$) reduced the rate of fat oxidation, volatile base nitrogen formation and microbial

growth during storage period. Control air packed samples were sensorily acceptable up to 10 days of storage and vacuum packed samples were acceptable till 12 days of chilled storage. The samples packed with oxygen scavengers were acceptable till the end of storage period but the microbial counts limited the shelf life to 18 days indicating a significant shelf life extension of 8 days compared to control air packed samples.

Microbial studies revealed that oxygen scavengers altered the condition inside the package, there was a shift in micro flora from gram negative, particularly H_2S producers in control air packs to gram positive flora *B. thermosphacta*, and *Lactobacillus spp* in commercial oxygen scavenger packs. *B thermosphacta* was found to be the dominant bacterial group next to *Lactobacillus* in commercial oxygen scavenger packs. *C botulinum* toxin was not detected in any of the samples during the storage period.

The effect of developed dual action sachets SAI, SAIFC and SAFS with CO_2 emission and O_2 scavenging capacity on the quality of yellowfin tuna was determined during chilled storage. It was then compared with samples packed with commercial oxygen scavenger, vacuum pack and air pack. All three developed chemical combinations were able to reduce the oxygen concentration to less than 1% within 24 hours. The oxygen concentration was completely removed within second day and the oxygen free condition was maintained till the end of storage in the case of samples packed with developed sachets. Whereas, the commercial oxygen scavengers reduced the oxygen content inside the packs to 0.01% within 24 hours of storage. All three chemical combinations emitted CO_2 during storage and maintaind the maximum CO_2 concentration till the end of storage. It was observed that developed dual action sachets were significantly effective in

increasing the shelf life of yellowfin tuna compared to control air packed samples. Freshness indicators like K value, Ki value, H value, G value, P value and Fr value of yellow fin tuna during chilled storage showed significant ($p<0.05$) difference between samples packed in control air and active packed samples. Yellowfin tuna chunks packed in air and vacuum exhibited more biogenic amine values than in active packed samples. Samples packed with air, vacuum and developed sachets with SAI, SAIFC, and SAFS crossed the microbial acceptable limit on 9^{th}, 12^{th}, 18^{th}, 20^{th}, 22^{nd} and 18^{th} days of storage respectively. This indicates a shelf life of 6-9, 9-12, 15-18, 18-20, 20-22 and 15-18 days for control air, vacuum, commercial oxygen scavenger and developed sachets SAI, SAIFC and SAFS respectively. The sensory score of samples packed with air and vacuum packs correlated well with the microbial count during storage. However, the sensory score was not in accordance with the microbial score of all active packed samples. The physicochemical and microbial studies of yellowfin tuna chunks stored under various packaging treatments showed that among the developed chemical combinations, SAIFC exhibited the best active packaging functions to maintain the quality and shelf life of yellowfin tuna during chilled storage.

The novel packaging technology presented in this study is simple, economic and cost effective. This study indicates that by using this technique, yellowfin tuna can be preserved safely up to 22 days while maintaining the acceptable quality characters. This could be of great importance in the future especially in the sashimi and chilled fish market which depends mainly on the freshness of tuna meat. Moreover adoption of this technology is economical as expensive equipment required for vacuum packing and MAP can be avoided.

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APPENDICES

Appendix I

Sensory evaluation score sheet

Assessor …….

Date……

(Please score the sample characteristics by placing the relevant score)

Sensory Attributes	Sample I	Sample II	Sample III
Appearance			
Color			
Odor			
Flavor			
Texture			
Taste			
Overall acceptability			

Please score the sample characteristics according to the following scale

Quality grade description	Score
Like extremely	09
Like very much	08
Like moderately	07
Like slightly	06
Neither like nor dislikes	05
Dislike slightly	04
Dislike moderately	03
Dislike very much	02
Dislike extremely	01

Appendix II

Publications

- K. B. Biji, C. N. Ravishankar, C. O. Mohan, and T. K. Srinivasa Gopal (2015). Smart packaging systems for food applications: a review Journal of Food Science and Technology. DOI 10.1007/s13197-015-1766-7
- K.B. Biji, C.N. Ravishankar, R. Venkateswarlu, C.O. Mohan and T.K. Srinivasa Gopal (2016) Biogenic amines in seafood: a review. Journal of Food Science and Technology. DOI 10.1007/s13197-016-2224-x
- K B Biji, K R Remya Kumari, K A Anju.,Suseela Mathew, C N Ravishankar (2016). Quality characteristics of yellowfin tuna (*Thunnus albacares*). Fishery Technology. 53: 313-319.
- Presented a paper entitled 'Shelf life extension of yellow fin tuna (*Thunnus albacares*) by the application of active oxygen scavengers during chilled storage' under Indian Science Congress YOUNG SCIENTISTS AWARD PROGRAMME of 2015-16 held on Jan 04, 2016.
- Biji, K.B., Ravishankar, C.N., Mohan C.O., Lalita, K.V., and Srinivasa Gopal, T.K (2013) Application of oxygen scavengers for the shelf life extension of yellowfin tuna (*Thunnus albacares*) during chill storage (International symposium on Greening Fisheries -Towards Green Technologies in Fisheries 2013)