Thermal Processing of Smoked Yellowfin Tuna (*Thunnus albacares*) in Flexible Pouches

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by

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CERTIFICATE

This is to certify that this thesis entitled "THERMAL PROCESSING OF SMOKED YELLOWFIN TUNA (*THUNNUS ALBACARES*) IN FLEXIBLE POUCHES" embodies the original work done by Ms. Bindu. J, under my guidance. 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 any other university or Institution. She has also passed the Ph.D qualifying examination of the COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY, Cochin held in October 2006.

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DECLARATION

I, BINDU.J. do hereby declare that the thesis entitled "THERMAL PROCESSING OF SMOKED YELLOWFIN TUNA (*THUNNUS ALBACARES*) IN FLEXIBLE POUCHES" is a genuine record of bonafide research carried out by me under the supervision of Dr. T. K. Srinivasa Gopal, Principal Scientist and Head, Fish Processing Division, Central Institute of Fisheries Technology, Cochin and has not previously formed the basis of award of any degree, diploma, associateship, fellowship or any other similar titles of this or any other university or Institution.

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Dedicated to
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Introduction

1.0 INTRODUCTION

Tunas are among the largest and most specialized and commercially important of all fishes (Collete & Nauen, 1983). They are the fourth major internationally traded fish commodity and contribute 7.6% of the international fish trade in value terms (Thomas, 2008). The Indian Ocean contributes 19% of the world tuna catch (Pillai & Ganga, 2008). Tunas are found in temperate and tropical waters and are distributed all around the world. Tuna and related species belong to six genera of the Family Scombridae. World trends in fishing, processing and trading of tuna are influenced by a number of environmental and socio-economic factors. Fishing activities are influenced by factors such as resource availability, water temperature, current, season and operational costs, whereas processing depends on availability and quality of raw material, product formulation and product costs. The three major species of tropical tuna caught in the Indian ocean are skipjack, yellow fin and big eye. The principal markets for tuna are Japan, USA and the European Union countries. The major commodities traded are sashimi, canned, chilled, frozen and smoked products.

The contribution of India towards global production of tuna is negligible. The potential of the Indian Exclusive Economic Zone is estimated at 2.78 lakh tonnes (Pillai & Jyothi, 2007). In India, tuna fishing is in a transition stage with artisanal activity still remaining as the major production method. Though traditional fisheries for tuna existed in the Maldives and Lakshadweep Islands for more than 100 years, the industrial fishing activity in the Indian Ocean commenced only in the fifties as tuna long line fishery and was followed in the mid eighties by purse seine fishery. However the motorization of traditional crafts and adoption of new technologies for catching tuna has substantially improved the catches. In India, tuna fisheries are concentrated mainly on the coastal region while offshore tuna fishery resources are yet to be commercially exploited. The tuna exports from India have increased from 16,627 t in 2005-2006 to 23,778 t in 2006-2007. Tuna export realization grew from Rs 693.1 million in 2005-2006 to Rs 1303.8 million in 2006-2007 (MPEDA, 2007). Chilled tuna is the highest unit value earning item, while larger quantities of tuna are exported in frozen form. Major form of chilled tuna exported is whole tuna, yellow fin loins and gutted tuna.

The main internationally traded tuna forms are raw material for canning (fresh, frozen and pre-cooked loins), tuna for direct consumption (fresh, chilled and frozen) and canned tuna (solid pack, chunks, flakes and grated). Sashimi graded tuna is a delicacy in Japan and is made from extremely fresh tuna. Blue fin, big eye and yellow fin are the most preferred species for sashimi, whereas skipjack and albacore are preferred for canning. Canned products are packed in oil, brine, spring water or sauce. The principle producers of canned tuna are Thailand, USA and Spain. In 2005, 82% of tuna was consumed as canned product and 18% as fresh product. Japan consumes 78% of fresh tuna involved in the world trade. (Gopal *et al.*,2008).

There are numerous health benefits of eating tuna. It has high content of Omega-3 polyunsaturated fatty acids. The consumption of such fishes is believed to decrease the risk of heart diseases, reduce cholesterol, regulate blood pressure, prevent arteriosclerosis and have other health benefits. Tuna also contains minerals, such as phosphorous which is important for the nervous system and iodine which is conducive for balanced growth. It also contains vitamins like niacin and vitamin B_{12} for cell growth and proper metabolism of fatty acids and cholesterol. In spite of these benefits there are some risks associated with the consumption of tuna. The major risk in consumption of improperly handled tuna is histamine poisoning. Histamine is produced mainly due to the bacterial action of the amino acid histidine by the enzyme histidine decarboxylase and is correlated with temperature abuse and improper handling and storage. Another risk is the presence of methyl mercury in the tuna fish. Mercury from the environment gets converted to methyl mercury which gets absorbed into the fish. The fish tend to accumulate the mercury which is found to have a harmful effect on infant and unborn babies.

Proper handling of tuna is required to get a finished product with good quality. Tuna should be harvested with least stress to avoid the accumulation of lactic acid in the flesh so as to avoid the onset of rigor mortis and subsequent spoilage. The harvested tuna must be killed immediately and chilled onboard. Chilling may be by simple icing or using chilled seawater or refrigerated seawater. The fish should be held throughout at 0-1°C. Tuna is frozen onboard at - 20 to -30°C for

canning purpose and at -50 to -70°C for raw consumption. Tuna frozen at ultra low temperatures can be stored up to 2 years without much quality changes. Value added products from tuna include Sashimi grade tuna and sushi products which fetch a very high price in the market. Sashimi grade products include tuna loins, saku block, steaks and poke cubes. Canned tuna is the major commercial tuna product which is internationally traded. In India canned tuna is produced in very minor quantities and is mainly for the domestic markets. Several dried products like *'Mojama'* in the Mediterranean, and smoked *'Katsuobushi'* in Japan command a high price and demand in the trade. Smoked and dried tuna called '*masmin*' is a delicacy in the Lakshadweep islands and certain areas of the mainland. Skipjack tuna is utilized for the production of this product. Fishing and preparation of this product is the main source of livelihood for the inhabitants of these islands. Frozen products include whole tuna frozen, tuna fillets, tuna loins and precooked loins. Most of this is used for reuse in the canning industry. Other value added products from tuna include pickled tuna, tuna jerky, tuna biriyani, tuna sausage, tuna shaving, tuna paste, battered and breaded products from tuna etc. Tuna processing waste is also utilized to produce by products like protein hydrolysate, tuna silage, PUFA, tuna orbitals, tuna gelatin, tuna bone powder, tuna viscera powder, tuna skin leather etc.

Packaging plays a very vital role in today's society; it surrounds, enhances and protects the goods we buy right from processing through handling and storage to the final consumer. Food is packaged to preserve its quality and freshness, and also add appeal to consumers and to facilitate storage and distribution. Seafood scenario the world over is witnessing vast changes. Value addition and diversification to satisfy the ever changing and diverse demands from the importing countries as well as demand from urban markets are major challenges faced by the Indian fish processing industry. The consumers increasingly demand convenience food products that are of high quality, taste, appearance and nutrition. These products should be prepared with minimum preparation time and should be in the ready to cook or ready to eat forms. Increase in expendable income, increase in number of working women, awareness of the different types of convenience products and overall improvement in standards of living have contributed to this change. Technological

upgradation and value addition have been instrumental in processing several products from fish and marketing them in overseas and urban markets.

Thermal processing has been used to achieve long-term shelf stability for a wide range of seafood products and is one of the most widely used methods for fish preservation. The main objective of the thermal processing is to produce a safe and high quality fish product at a price affordable to the consumer. Thermal processing is mainly done in cans. Owing to their convenience, long shelf life and economy, canned foods form a major segment of processed food market. Nicholas Appert, a French confectioner in 1809 invented the art of thermal processing. In 1920, Bigelow and Ball presented the first scientifically based graphical method for calculating minimum process conditions for safe sterilization. In 1923, Ball developed a mathematical model for determination of sterilization process which was followed by a nomographic method for process determination by Olson in 1939.The technology has evolved over the years and currently the main focus is on increased efficiency in energy utilization and production, easy handling, more attractive packaging and better sensory quality (Durance *et al.*, 1997). Attractive packaging materials are available to present various products. The success of thermal sterilization necessitates balancing the beneficial and destructive influence of heat on the desirable characteristics of foods. Several changes occur in food during thermal processing of which some are desirable and some are undesirable. The heat treatment destroys the pathogens and spoilage enzymes that would bring about spoilage of the fish whereas nutritional quality is brought down by processing. The surface of canned meats and other solid-packed products may be darkened by contact with the inner surface of the hot can. Studies have shown that thermal death rates of bacteria generally proceed much faster with increased temperature.(Stumbo, 1973) Hence it is necessary to increase the rate of heat transfer into the food so as to reduce process time and maximize the retention of quality factors to get a good product. Thin profile utilizes the concept of larger surface area for rapid heating and cooling of foods packed in retort pouches or thin profile containers. Retort pouch packaging is of recent origin but have gained equal importance due to several reasons over the metallic cans. The retort pouch is a flexible, laminated package that can withstand thermal processing and has the

advantages of cans as well as flexible packages. The pouches show improved heat transfer and reduced process time and promote better quality. Thermal process parameters such as heating rate index (fh), heating lag factor (jch) and cooling lag factor (jcc) for the product help in determining the process time and optimal conditions for heat sterilization. However, these parameters are influenced by factors like mode of agitation, type of heating medium, temperature, rotational speed, headspace, can size and shape, product shape and size and carrier fluid viscosity (Sablani & Ramaswamy,1995; 1996 and Ramawamy & Sablani,1997a).

Smoking is another traditional method for preservation of fish and is widely practiced in the hilly, tribal and Lakshadweep islands of India. Major portion of the internationally traded smoked fish is prepared by cold smoking (temperature less than 30°C) by using different smoke flavourings. In tropical countries like India fish is preserved by hot smoking in traditional kilns at higher temperatures (more than 60°C). Masmin from skipjack tuna is the most important commercially available smoked fish product in India. It is the loin which is smoked and dried using coconut wood and husks. However, this product is very hard and represents a piece of wood, dry in texture due to very low moisture content. Though the product is relished by the traditional users there is a lot of inconvenience to the new consumers during usage.

Hence studies to develop an alternate product from tuna capable of storage at ambient temperature for more than a year, with convenience of use and having the same flavour of smoke of coconut husks have been attempted. Since the tuna used in the studies is yellow fin, this study will help in utilizing the smaller fish resources which otherwise will not be used as sashimi grade. The technology and trade of sashimi and chilled products from tuna is yet to catch up in the Indian sub continent, and at present the available catch can be utilized to develop value added products like smoked tuna in retortable pouches, which are ready to use and can be made readily available in the market. This is the first time that such a product has been developed in India by using locally available wood for flavour and having an extended shelf life of a year at ambient temperature.

The objectives of this study are:

1. To develop high moisture smoked tuna products with increased shelf life at ambient temperature storage.
2. To identify and standardize suitable indigenously available wood as the source of smoke flavour.
3. To identify appropriate packaging material for the smoked and thermal processed products
4. To standardize the heat processing parameters of the product in different packing media.
5. To investigate the heat penetration characteristics of the product in different types of flexible pouches
6. To investigate the effects of rotational speed on the heat penetration characteristics of smoked tuna products packed in different medium
7. To investigate the effect of processing on the different smoke components during thermal processing.
8. To determine the shelf life by studying changes in biochemical, sensory and textural characteristics of the developed products during ambient (28 ± 2°C) and accelerated temperature storage (37 ± 2°C).

Review of Literature

2.0 REVIEW OF LITERATURE

2.1. Packaging

Food packaging is crucial to our modern food distribution and marketing systems. Without protective packaging, food spoilage and wastage would increase tremendously. The advent of modern packaging technologies and new methods of packaging materials made possible the era of convenient foods. In the past the packaging emphasized the expectations of the producers and distributors but has now shifted towards the consumer since they are becoming more demanding and aware of the choices available (Piergiovanni, 1991). A food package usually provides a number of functions in addition to protection. Convenience packaging for consumers requires a shape and size of containers that allow for easy opening, pouring, serving, carrying, reclosing and storage.

Fish is one of the most perishable of all foods. The best package cannot improve the quality of the contents and so the fish must be of high quality prior to packaging (Fujita,1990). Different products have different packaging requirements and it is important to choose suitable packaging material accordingly. It is important to know the intended storage conditions of the product, i.e., temperature, relative humidity and expected shelf life. This is why multilayered plastics are very popular since properties of different films can be effectively used (Mueller, 1991). The basic function of food packaging is to protect the product from physical damage and contaminants, to delay microbial spoilage, to allow greater handling and to improve presentation. Emergence of thermo process- resistant films from the petroleum industry and advances in the thermo processing procedures have given rise to retortable packaging, which now plays a very important role in food packaging (Yamaguchi, 1990). Flexible retort pouch and semi rigid trays are used for thermal processed products (Fujita, 1990).

2.1.1. Retort Pouch

The concept of pouch as a container was developed by the US Army Natick Laboratories and a consortium of food packaging companies in the early 1960s (Herbert & Betteson, 1987). The technical and commercial feasibility of using retort pouches for thermo-processed products have been proven by (Hu *et al.*, 1955). In 1967, Chinese dumplings and curry were packed in aluminum foil containing retortable pouches and marketed. In the year 1968-69 commercialization of curry in foil free and aluminum foil containing pouches were undertaken and this started the era of retort pouches in Japan (Tsutsumi, 1972). The boil in bag concept of warming the food before consumption gives an edge for pouches over cans (Arya, 1992). Retort pouched products are shelf stable ready to eat products which can be used as per the convenience of the consumer (Devadasan, 2001 and Rangarao, 2002). The most comprehensive work on flexible packaging for thermal processed foods was prepared by Lampi (1977). Heat sterilized low acid solid foods in pouches created a new segment within the canned foods category (Brody, 2003). Sara *et al.*, (1989) studied the effect of increased over pressure levels, entrapped air and temperature on the heat penetration rates in flexible packages. Sacharow, (2003) did market studies in USA and Europe and reported a bright future for retortable pouches.

2.1.2. Components of retort pouch

The use of retort pouch for heat processing of food was reported earlier by Hu *et al.*, (1955) and Nelson *et al.*, (1956). Tripp, (1961) reported the feasibility of retort pouches for producing different food products. The US Military developed a packaging material made up of 75 µ vinyl / 9 µ foil / 25 µ polyester (McGregor, 1959) for use as retort pouch. Several authors have discussed in detail different properties of flexible packaging materials required for thermal processing of food products (Heidelbaugh & Karel, 1970; Nieboer, 1970; Rubinate, 1964; Szczeblowski, 1971; Schulz, 1973 and Tsutsumi, 1974). The three or four layer retort pouches consists of an outer polyester layer, a middle aluminum layer and an inner cast polypropylene layer (Griffin, 1987). Nylon is also added as an additional layer or is substituted for the aluminum layer to give

additional strength in a four layer pouch. Aluminium foil is the barrier layer which gives the product a longer shelf life (Rangarao, 2002). Polypropylene has a high melting point of about 138°C and is used as the inner layer to provide critical seal integrity, flexibility, strength and taste and odour compatibility with a wide range of products (Shorten, 1982). The different layers are held together with adhesives which are usually modified polyolefins such as ethylene vinyl acetate (EVA). Martin, (1966), Goldfarb, (1970) and Nieboer, (1970) have given an account of the different adhesives used for retort pouches. Taylor, (2004) has reported the possible use of liquid crystal polymers, which have superior oxygen and water vapour barrier properties compared to other polymer films. Some pouches contain polyvinylidiene chloride, ethylene vinyl alcohol or nylon instead of the aluminium layer to permit viewing of the product. These are foil free laminated materials. These plastics are good barriers to oxygen molecules but are not complete barriers and therefore the shelf life is reduced (Jun *et al.,* 2006). Nowadays retort pouch containing a coating of nano particles like silicon dioxide or aluminium oxide in addition to the other mentioned layers are commercially available in the market. These pouches have good barrier properties and are comparable to aluminium foil pouches. The different types of retort pouch and the layers which make up the retort pouch are given in Table 1.

2.1.3. Advances in retort pouch processing

Stephen and Wiley, (1982) compared general method and Ball's formula method for process calculation and found that Balls method was more suitable. Process determination for conduction- heated foods in retortable pouches was reported by Bhowmik and Tandon, (1987). Lebowitz and Bhowmik, (1989) reported the heat transfer coefficient for retortable pouches. Skipnes, (2002) studied heat transfer of vacuum packed farmed mussel subjected to different pressure, holding time and variations in vacuum in retort pouches. Evaluation of quality of chum salmon processed in retort pouches and metal cans to a equivalent lethality showed that the pouch products were firmer, drier, chewier than the canned ones (Durance & Collins, 1991). This is in agreement with the observations of Skipnes *et al.,* (2002) on Alaska Pollack, shrimps and rainbow

trout where the pouched samples had a firmer texture and lighter color. Heidelberg and Karel, (1970) reported the quality changes in pouched food.

Table 1. Properties of material used for retort pouch manufacture (Gopal, 2007)

	Materials	Advantages	Disadvantages
Outer layer	Polyester	Good heat resistance and Reverse colour printing	Probability of having pin holes high
	Biaxially oriented nylon	High strength	Shrinks during heat processing
	Non oriented nylon	High strength and very good heat resistance	Difficulty in quality printing
	Biaxially oriented polypropylene	High strength and sealing	Develops shrinkage during heat processing; has the tendency to curl
Middle layer	Aluminium foil	High barrier	Pin holes, has opaque colour, poor heat sealing quality
	Polyester	High strength, heat resistant	Pin holes
	Nylon	High strength and Impact resistance	Expensive
Inner layer	High density polythene	Good impact resistance High sealing property; cheap	Poor heat resistance, translucent
	Non-oriented polypropylene	Transparent, good heat resistance	Poor impact resistance

In the sensory acceptability, pouched products has a better overall acceptability than canned products since there is less impairment to the texture and colour due to the reduced exposure to heat (Durance & Collins, 1991). Dymit, (1973) reported that shrimp in pouches were superior in flavour and colour compared to canned products. Synder and Henderson, (1989) found that retort pouches reduced times for conduction heating products but in a liquid medium

the agitated cooking of cans required less processing time than the pouches. Retort pouch products from Tuna and Salmon have a successful market in the USA. Brody (2000); Balange (2002) and Chai *et al.*, (1984) observed that oysters could be processed with less severe treatment when packed in retort pouches and they had a extended shelf life. Heat penetration was faster in fish balls packed in curry in retort pouches with good sensory properties (Balange, 2002). Adams and Otwell, (1982) reported that products like fish, shrimp, crab and meat processed in pouches were superior to canned products. Kluter, *et al.*, (1994) studied shelf life of cling peaches in retort pouches and Taiwo *et al.*, (1997) studied cowpeas in tomato sauce and found that retort pouches are economically feasible as an alternative to the cans.

2.1.4. Benefits of retort pouches

Pouches provide better quality product due to rapid heat penetration, shelf stability without refrigeration, convenient end use preparation, and compact storage characteristics before and after packaging, easier opening and disposal (Ramaswamy & Tung, 1988). These pouches are able to withstand high sterilization temperatures including HTST operations (Awuah *et al.*, 2007). In addition to this, the pouches should be able to withstand cooling temperatures of around 40°C under counter pressure, to avoid opening of heat weakened seals (Brody, 2002). Retort pouches can withstand thermal processing and combines the advantages of the metal cans and boil in bags (Gopal *et al.*, 1981). Natural convection plays an important role in the heat transfer in liquid products and conduction and convection in more solid products. Food packed in pouches have the advantage of improved quality , reduced weight, improved carrying ease and an energy saving advantage over the equivalent canned ration in military (Steffe *et al.*,1980). The advantages of retort pouches technology *vs* conventional metallic canning and advantages of retort pouches versus metallic cans are given in Tables 2 and 3 respectively.

Table 2: Retort pouch vs conventional metallic canning (Venugopal 2006).

Features	Retort Pouch	Metal Can
Feasibility	Highly suitable for delicate products such as seafood, sauces	Good for products having tough texture such as beef, pork etc
Product development	Slower filling, thermal processing more complex	Convenient product line including filling and thermal processing
Sterilizationtime	Less	More
Product quality	Superior in quality , with more natural colour , flavor and texture	Intense cooking results in loss of natural sensory attributes
Shelf life	Comparable with canned products	Comparable with retort pouch products
Convenience in handling	Less weight , needs less storage space	More weight, requires more space for storage
Convenience in consumption	Can be easily opened by tearing across the top notch in the side seal or by cutting with a scissors	Requires a can opener
Capital investment	Higher capital investment	Medium level of capital investment
Marketing	Trade and consumers need to be familiarized with handling the pouch	Established technology and hence minimum consumer attention needed

Table. 3. Advantages of retort pouches *vs.* metal cans (Jung *et al.*, 2006)

Features of retort pouch packaging	Benefits of retort pouch
Reduced cooking time	Better taste, nutritional value and faster turn around time
Complete product evacuation	Improved product yield and consumer value
Reduced bulk and weight	Lower transportation and storage costs
Environmental friendly	Less waste and fossil fuel consumption
Package differentiation and larger shelf display	Increased sales
Package durability	Eliminate cuts and promotes employee safety
Rotogravure printing	More attractive graphics on packaging
Package durability	No dented cans

2.1.5. Retort pouch in India

In India the research on retortable pouches started in the 1970s. General information on retort pouch processing and use of retort pouch as an alternative to cans has been reported by Gopakumar and Gopal, (1987). Flexible pouches of three layer configuration, which can perform the packaging functions of boil in bags as well as cans, were identified. (Gopal *et al.*, 1981); Gopal *et al.*, (1998) and Vijayan *et al.*, (1998). Girija *et al.*, (1996) reported the processing of various fish curry products in retortable pouches. A number of ready to serve fish products like ready to serve fish curry (Gopal *et al.*, 2001), Kerala style fish curry (Vijayan *et al.*, 1988) Rohu curry (Sonaji *et al.*, 2002) , Seerfish curry (Gopal *et al.*, 2002; Ravishankar *et al.*, 2002), Seerfish moilee (Manju *et al.*, 2004), Ready to eat mussels (Bindu *et al.*, 2004), Prawn *Kuruma* (Mohan *et al.*, 2005), Grey clams (Bindu *et al.*, 2007), and Etroplus curry (Pandey *et al.*, 2007) have

been processed in retort pouches. Ali *et al.*, (2006) compared the properties of indigenous and imported packaging material and studied the heat processing of fishes in retortable pouches. Madhwaraj *et al.*, (1992) reported that spoilage in the flexible pouch is due to contamination of seal area.

2.1.6. Major physical properties of retort pouch

2.1.6.1. Thickness

The thickness of the pouch has a direct influence on the heat penetration characteristics and product quality. Non uniform thickness can affect the machine performance, product protection and integrity of the packages (Hemavathi *et al.*, 2002). The acceptable limit for variation in thickness of individual layers is ± 2 µm (inner ply) or 10 % of the value (Lampi, 1977; 1980). The thin profile helps in rapid transfer of heat to the inner regions unlike in cans and glass bottles. About 20-30% reduction in process time was observed by Simpson *et al.,* (2004) for vacuum packed mackerel in retortable pouch and processed in steam /air mixture at 116.8°C.

2.1.6.2. Barrier Properties

Plastic films may have pin-holes which under normal conditions are unlikely to occur when they are laminated (Yamaguchi, 1990). It has been confirmed that even a single layer film does not allow the microorganisms to pass (Lampi,1977). Hence the problem lies in careless handling during the manufacture of the products and during handling and exposure in transportation and storage.

a) Oxygen transmission rate (OTR)

Oxygen transmission rate is of great importance in the packaging of processed foods to exclude headspace oxygen (Kumar, 1994). The OTR in imported and indigenous pouches tested as per ASTM D 1434 was practically nil for the three layer pouches (Vijayalakshmi *et al.,* 2003).

b) Water vapour transmission rate (WVTR)

The retort pouches consist of multiple films laminated together. Since the pouches do not have any pin-holes under normal manufacturing conditions the WVTR rate is practically nil. Contamination by penetration of water contaminated with microorganisms is of major concern during the cooling process. In retort foods, the wide sealing width does not allow the entry of water into the pouch from the seal area. The cooling water should be clean for maintaining a quality product (Yamaguchi, 1990)

2.1.7. Physical strength for packages

2.1.7.1. Tensile strength and elongation at break

Strength is required to protect the product during processing, distribution and retail handling. Polyester and nylon are commonly used as oriented films since they are generally stronger than unoriented films (Ghazala, 1994).

2.1.7.2. Heat seal strength

The heat seal strength is an important property for packaging integrity and to provide shelf life. Polypropylene (PP) can tolerate temperatures upto 130°C and can only be reheated in the microwave (Forshaw, 1990). PP has good heat seal strength and as such it is used as the inner layer for heat sealing.

2.1.7.3. Bursting strength

Low values of bursting strength indicate easy delamination of the layers during thermal processing which results in physical destruction of the pouch and reduction in barrier properties (Vijayalakshmi *et al.*, 2003).

2.1.7.4. Residual air

The presence of residual air is associated with seal integrity and influences heat transfer, sterilization effect and product quality (Yamaguchi,

1990). Different techniques for the removal of residual air have been proposed but have limitations when applied on a commercial basis (Mayer and Robe, 1963; Heid,1970 and Schulz and Mansur, 1969). The stretch method is applied for pouches of standard sizes in Japan (Tsutsumi, 1972) and no particular problem has been experienced with the residual air (Yamaguchi *et al.*, 1972). 15 ml or more residual air in the case of standard size pouch markedly interferes with the heat transfer and affect the sterilization process (Yamaguchi *et al.*, 1971)

2.1.7.5. Overall migration test

Plastic in the finished form contain non polymeric components (mainly additives) which may leach out into the packed food when it come into direct contact with the food (Vijayalakshmi *et al.*, 2003). The selection of suitable packaging material for food contact application is decided on the basis of the physical, mechanical, barrier and performance properties of the films (Iyer, 1992). These may contaminate the food and be harmful to the human body. Since the migration is inevitable, different countries have prescribed limits for these extractible substances. As per Indian Standard the limit for finished materials is 10mg/dm^2 or 60 ppm. Vijayalashmi *et al.,* (1992) reported higher migration from retort pouches into n- heptane, than into water. This may be due to the structural similarities of n- heptane with the contact cast PP layer

2.1.7.6. Shelf life of retort pouch foods

The shelf life of a retorted food product is about 2 year for an aluminium foil pouch (Szczeblowski,1971 and Thorpe & Atherton,1972) 2-3 months for a barrier type non foil pouch and 1-2 months for a non foil retortable pouch (Yamaguchi , 1990). Studies on hamburgers packed in retort pouches showed that sensory characteristic depends on the oxygen permeability of the packaging material (Ishitani *et al.*, 1980). Komatsu *et al.*, (1970) have studied the effect of headspace gas on thermal processing and shelf life of processed products. Dymit, (1973) reported that after 8 years shrimp in a foil laminate was superior in flavor and color to the canned item. Pouches using aluminium foil have a longer shelf life with respect to quality. Vijayan *et al.*, (1998) reported that curry

processed in indigenous retort pouches could be kept in good condition for 24 months even though there was slight transmission of water and oxygen.

2.2. Smoking preservation of fish

2.2.1. Smoking of Fish

Smoking is one of the oldest methods of preservation of fish and it combines the effects of salting, drying, heating and smoke components (Bligh *et al.*, 1988). The preservative action is mainly by lowering the water activity and by the deposition of the smoke components produced by the thermal degradation of sawdust or wood. A wide variety of organic constituents such as phenolic, carbonyl and organic acids are present in the smoke (Asita & Campbell, 1990). These compounds, along with the low water activity and applied heat inactivate autolytic enzymes and retard the growth of spoilage microorganisms (Gilbert and Knowles, 1975; Dillon *et al.*, 1994 and Sikorski, 1994). Smoke may mask the spoilage changes and affect the relationship between sensory and microbiological changes. The smoke is usually concentrated on the surface of the skin and penetrate not more than 1 cm under the skin during storage (Hansen, 1995 and Sikorski, 1994). In the underdeveloped and developing countries smoking is used as a method of fish preservation whereas in the developed countries, it is practiced mainly to impart colour of a particular wood rather than for preservation (Wheaton & Lawson, 1985). In traditional practices, the processors use unsophisticated equipments and has little control over the smoking parameters whereas in the present advanced techniques, knowledge of the chemical and biological properties of the raw material combined with the use of smokehouses with precise control of smoke density, temperature, humidity etc facilitate the production of smoked products with desirable, predictable nutritional and sensory quality with appropriate shelf life (Doe, 1998). The use of controlled heating parameters makes it possible to calculate the bacteriological lethality of the smoking process (Sznajdowska, 1983). Cold smoking is a process where the dry bulb temperature is kept below 30°C for the entire process (Horner, 1992 and Regenstein & Regenstein, 1991). Hot smoking is simply a heating process that fully cooks the fish to 160 °F or higher (Raab & Hilderbrand.Jr., 1993). There is a

distinctly different flavour, texture and appearance between fish prepared by hot and cold smoking (Whelan, 1982). Traditional smoke-preserved fish are being replaced by light smoke flavoured fish products (Horner, 1992). In light smoking, the fish is light salted, light smoked to get a product of attractive appearance, odour, colour and flavour.

2.2.2. Smoke generation technology

Smoking is generated by burning wood or sawdust or a combination of both. Wood burns quickly and gives a hotter fire with a lesser smoke which may char the fish. Sawdust on the other hand burns slowly and unless the draught is very strong it will not catch fire. Hardwoods are preferred to softwoods since soft woods contain resins which impart a bitter taste to the product. A combination of wood shavings and sawdust can also be used in the case of hot smoking. The choice of wood depends on the flavour required (FAO, 1970). Oak, Mahogany, Cedar, Hickory, Teak and Mesquite are the commonly used woods for smoke production. The wood and sawdust should be dry and free of moulds and preservatives since the smoke may carry the harmful substances like moulds and other chemicals to the fish which may make the fish dangerous to eat.

2.2.3. Smoke and its components

The preservation of smoked fish is mainly due to the different stages in the smoking process and the different components present in the smoke. The initial process of brining the fish before smoking helps in removal of water and thereby reducing the water activity. This inhibits the growth of many pathogens and spoilage microorganisms. The smoke helps in surface drying of the fish and as a result there is a physical barrier for the microorganisms to proliferate into the fish. Smoking at high temperatures cooks the fish and removes the moisture inside the fish and lowers the water content thereby retarding the spoilage. Smoke deposits antioxidant compounds like phenols which delays the auto oxidation of the unsaturated fish lipids and avoids rancidity. Antimicrobial compounds like phenols, aldehydes, nitrites etc. also enhance the preservative action of smoke (Balachandran, 2001). Wood smoke is complex systems consisting of disperse

and particulate phases. The disperse phase contains vapors which imparts the characteristic colour, flavour and preservative properties to the smoked fish. The particulate phase acts as a reservoir for compounds having a high vaporization temperature. Between the particulate and vapour phase of the smoke there is a dynamic equilibrium that changes with fluctuations in temperature, density of smoke, velocity of smoke and absorption of smoke components by the fish surface (Tilgner *et al.*, 1965). Smoke contains more than 400 chemicals of which about 200 have been identified. The commonly found chemicals are carbonyls, organic acids, phenols, organic bases, alcohols, hydrocarbons and gases such as CO_2, CO, O_2, N_2 and NO (Daun, 1979). Smoke contains antioxidative and antimicrobial properties. The antioxidative properties are due to the presence of high boiling phenols like 2, 6, dimethoxyphenol, 2, 6 dimethoxy-4-ethylphenol, 2, 6, dimethoxy-4-ethylphenol, guaicol, etc (Wheaton & Lawson, 1985). Low boiling point phenols show weak antioxidative properties (Tilgner *et al.*, 1965, Radecki *et al.*, 1975). According to Daun, (1979) the method of smoke generation influences the antioxidative properties of smoke, with a smoldering type fire producing better properties.

2.2.4. Antimicrobial or bactericidal properties of smoke

Antimicrobial properties of smoke are mainly influenced by chemical components of the smoke and the treatment of the fish prior to smoking. Carboxylic acids, phenols, aldehydes and ketones have a distinct role in reducing the bacteria. Vegetative forms of bacteria are more sensitive to wood smoke whereas spores are more resistant. The temperature of smoke generation influences the antimicrobial properties of smoke. Fredheim *et al.*, (1980) found that smoke condensates produced at 350°C inhibited the growth of *S. aureaus, E.coli and S.cerevisae* at lower concentration than the condensates that were produced at higher temperatures. The effect of pasteurization in hot smoking depends on the composition of fish, the salt content, humidity, temperature, type of wood and duration of the process and on the interaction of the smoke components (Doe *et al.*, 1998). The important factor determining the multiplication of bacteria in smoked fish is the temperature of storage. The microbial hazard of the formation of toxin by *Clostridium botulinum* type E can be

avoided by sufficient heating of the product followed by chilling and maintaining the storage temperature of the product below 3°C (Sikorski & Kolodziejska, 2002). As per FDA requirements, the thickest part of the fish should have maintained a temperature of 82°C for 30 minutes and the NaCl content in the water phase of the meat is 3.5%. To get a superior quality smoked fish product the salt and heat used should be mild and hence the antibacterial effect of smoke is not very effective here. Moreover vacuum packed samples being anaerobic have a greater chance of harboring the organisms and hence the only protection from botulinum is by maintaining good manufacturing practice and keeping the product below 3°C and avoiding temperature abuse (Sikorski & Kolodziejska, 2002). Smoking effects a change in the bacterial flora from gram negative to gram positive microflora probably due to the sensitivity of the gram negative bacteria to the antimicrobial compound of the smoke. Hansen, (1995) and Leroi *et al.*, (2000) did not find any difference in the sensitivity of gram negative and gram positive organisms to smoke. Gancel *et al.*, (1997) isolated lactobacilli from vacuum packed salted and smoked herring and found all strains to be resistant to liquid smoke. Cold smoked products are not cooked and have to be refrigerated and hence post the risk of the presence *of L. monocytogenes* which can grow at low temperatures. Listerosis caused by smoked fish can be avoided by control of temperature in the range of 60-65°C as the ultimate core temperature necessary to ensure thermal inactivation of *L. monocytogenes*. (Kolodziejska *et al* ., 2002).

2.2.5. Antioxidative properties of smoke

Different types of wood components at certain levels have proven to contain antioxidative properties. Tilgner *et al.*, (1965) have demonstrated the antioxidant activity of phenolic compounds present in curing smoke. Smoke flavoring was found to have an inhibitory effect on the auto oxidation of lard (Chomiak & Goryn, 1977). Curing smokes obtained by three smoke generation methods was found to vary in its antioxidative properties (Tilgner & Daun, 1970). Smoke can be used to retard lipid associated rancidity in a wide variety of smoked foods (White, 1941). Toth and Potthast, (1984) fractionated wood smoke into acid, basic and neutral portions. They demonstrated that neutral portions contain a major portion of the phenolic compounds which had the best

antioxidative properties, whereas the acidic portion had minor antioxidative properties while the basic portion actually promoted lipid oxidation. Phenols are the primary antioxidant - related compounds associated with wood smoke (Daun, 1969). Most commonly used synthetic antioxidants like (butylated hydroxyanisole (BHA) and butylated hydroxytolulene (BHT) are phenolic in nature (Maga, 1988). Smoke flavorings ranging from 0.2 to 2 % levels have been found effective in antioxidant properties (Watts & Faulkner, 1954). Use of 10% of liquid smoke for antioxidant properties was actually found to be prooxidative in lard (Chomiak & Goryn, 1977). Radecki et *al.*, (1975) found that smouldering smoke generation results in strong antioxidative properties. The particle phase of smoke has more antioxidant properties than the vapour phase (Daun & Tilgner, 1977)

2.2.6. Effect of smoke on lysine content

Amino acids are easily lost during different stages of processing. In smoking the amino acid of major concern is the essential amino acid lysine. Lysine is available in small quantities and is a very reactive compound and can enter into many chemical reactions. Chen and Issenberg, (1972) have reported a loss in the total lysine content during smoking. Di Cesare *et al.*, (1980) have reported a loss in lysine content in fillets of *Sardine pilchardus.* Similar results were obtained by Petrichenko and Dolzhenko, (1981) in muscle proteins of Cyprinids and by Cattaneo *et al.*, (1983) in smoked *Salmo gardneri.* Dvorak and Vognarova, (1965) concluded that short smoking time did not influence lysine loss. Losses up to 44% was reported in uncured beef smoked at 65°C for 10 hour whereas samples processed without smoking lost up to 15% lysine indicating that smoking lowers the available lysine content when compared to other processing methods (Chen & Issenberg ,1972) .

2.2.7. Smoke and food colour

Carbonyls and certain phenols present in the wood smoke are responsible for the colour of the smoked foods. Colour, is important since it is the first sensory factor followed by aroma, flavour and texture that denotes the food acceptability (Maga, 1988). Carbonyl compounds present in the smoke are major contributors

to the colour formation in smoked meat. A series of non enzymatic reactions similar to maillard reactions occurs due to the interaction between the carbonyls in the smoke vapor and the amino group from the protein on the food surface (Chen & Issenberg, 1972; Gilbert & Knowles, 1975 and Ruiter, 1970). The most reactive carbonyls are glycolic aldehyde, methyl glycol, formaldehyde and acetol and the first two produces the most colour (Ruiter, 1970). Phenols with high molecular weight present in the vapour phase of the smoke also contribute to the colour formation in smoked foods. (Caurie *et al.*,1974). These phenols should have sufficient number of hydroxyl group to cross link protein at numerous sites through hydrogen bonding. Coniferaldehyde and sinapaldehyde are such polyphenols which can react to form colour, however their levels in food are usually low when compared to carbonyls (Chen & Issenberg, 1972)

2.2.8. Potential health concerns associated with smoke

Poly aromatic hydrocarbons (PAH) act as potent chemical carcinogen and hence their presence in smoked fish is of major concern. PAH compounds are formed during the thermal degradation of wood, especially when the wood is burnt with limited access to oxygen (Bartyle,1991). Smoke generation conditions can significantly affect the level of PAH in the smoke and smoked fish (Potthast, 1978; Steinig, 1976 and Steinig *et al.*, 1977). There is an increase in the PAH content with increase in temperature of smoke generation especially in the ranges of 400-1000°C (Toth *et al.*, 1972). The international agency for research on cancer (IARC) classified benzo (a) anthracene, benzo (a) pyrene and dibenzo (a,h) anthracene as probably carcinogenic to humans and benzo(b) fluoranthene and benzo(k) fluoranthene and indeno (123-cd) Pyrene as possibly carcinogenic to humans (US-EPA, 1984; IARC, 1987). Benzo (a) pyrene (BaP) is the leading carcinogenic compound which is accepted as the indicator of total PAH presence in smoked foods (Andelman *et al.*, 1970). PAH compounds when ingested into the human body interact with enzymes to form derivatives which are believed to be carcinogenic in nature. These are capable of forming covalent adducts with proteins and nucleic acids and initiate cell mutation which results in malignancy (Rogan *et al.*,1993; Stahl & Eisenbrandt, 1998 and IARC, 1987). Kangsadalampai *et al.*, (1997) concluded that the most potential mutagenicity

was observed in PAH fractions isolated from smoked fish treated with nitrates in an acid solution. The European commission, (2005) in its regulation 208/2005 limits the level of BaP content to 5 µg/kg in smoked meat, smoked meat products, muscle meat of smoked fish and smoked fishery products. And for liquid smoked flavored products the maximum permissible limit is 0.03 µg/kg as per directive 88/388/EEC. Preparation of heavy- smoked products in uncontrolled conditions typical of home smoked without the application of good manufacturing practices is far more dangerous than those produced in controlled conditions (Simko, 2002). Exposure to UV light degrades PAH compounds and forms oxidation compounds like aromatic alcohols, ketones, quinines and esters (Bernstein *et al.*, 1999). Toxicity of these compounds maybe even enhanced when compared to the original compounds (Bernstein *et al.*, 1999). The rate of deposition of PAH on fish depends on smoke characteristic like temperature, humidity, flow rate and density of smoke. The PAH content are higher for fish with a higher lipid content (Storelli *et al.*, 2003).

2.2.9. Textural changes in smoked fish

Heating results in protein denaturation or loss of solubility and brings about textural changes in the protein which is a major component of muscle foods (Maga, 1988). Smoking significantly lowers the myofibrillar and sarcoplasmic protein nitrogen fractions and increases the stromal fractions (Randall & Bratzler,1970). These changes result in cross linking of surface proteins resulting in a firm and stable crust which is texturally harder than the inner portion of the fish muscle. This skin like formation hinders the penetration of smoke components towards the interior portions of the flesh and thus results in uneven distribution of colour and flavour (Maga, 1988). The outer surface of the smoked meat was found to be rather dry and had a low water holding capacity probably due to the fact that the smoke components had reacted with the proteins by not permitting protein to hold large quantities of water. Therefore the texture on the outer surface of the material was rather dry and tough in comparison to the interior portion (Radetic *et al.*, 1982).

2.2.10. Brining and its effect on smoking

Brining helps in reducing the water activity and thereby increasing the antimicrobial effect of the smoke at higher temperatures. The reduction in water activity is related to the amount of salt that is used for the product. Salt used should be within the permissible limits so that it will not affect the palatability. In light smoked vacuum packed trout contaminated with *C botulinum* the least concentration of NaCl required for inhibiting toxin was 2% (Cann & Taylor, 1979). A minimum of 3% salt is required to inhibit *C botulinum* for 30 days at 10°C. Peterson *et al.*, (1993) found that neither 3% nor 5% water phase NaCl prevented the growth of *L monocytogenes* at 5 or 10°C. Inhibition of growth of the organisms were found at 6% water phase salt only and was found to be inversely proportional to the size of the population. Growth of *Staphylococcus aureus* on hot smoked Snoek was inhibited at 4°C when the water activity was 0.96 but multiplied at 24°C when the water activity was 0.94 (Theron & Prior, 1980).

2.2.11. Biogenic amines in smoked fish

Biogenic amines are low molecular weight organic bases that occur due to the microbial action during storage of foods products. These amines are formed and degraded as a result of normal metabolic activity and can be produced by the decarboxylation of amino acids (Halasz *et al* ., 1994). They are generated as a result of endogenous amino acid decarboxylase activity or by the growth of decarboxylase positive microorganisms under favourable conditions for enzymatic activity. (Evan & Malmberg, 1989). In human and animal physiological functions serotin, histamine and tyramine plays an important role and in plants spermidine, spermine and putrescine are important. Biogenic amines are of great concern in relation to food spoilage and food safety. Since microbial spoilage of food is accompanied by the increased production of decarboxylases, the presence of biogenic amines serves as indicators of bacterial contamination and food spoilage. Biogenic amine estimation is important from the point of view of their toxicity as well as indicators of the degree of freshness or spoilage of food (Halasz *et al.*, 1994). Histamine along with other amines like putresine, cadaverine may have a synergistic effect and hence efforts to optimize

production and storage conditions to secure low amine levels in food may be done (Smith, 1980). Other biogenic amines are less toxic than histamine, however nitrosoable secondary amines like agmatine, spermine and spermidine can form nitrosamines by reaction with nitrites and produce carcinogenic substances.

Histamine poisoning is also referred to as scombroid fish poisoning and is associated with the consumption of scombroid fishes like tuna, mackerel, saury bonito and seer fishes (Taylor, 1986). Non scombroid fishes like sardines, anchovies, herring, marlin etc have also been implicated in cases of histamine poisoning (Taylor, 1985). Tuna and other related species like mackerel, seer fish are found to contain high level of histidine which may be converted to histamine by microorganisms. The formation of histamine was dependent on the temperature of storage and microbial activity. Klausen and Lund,(1986) suggested that amine content depends on temperature and observed that at 10°C the amine contents were 2-3 times higher than at 2°C in both mackerel and herring. A positive correlation was found between increasing microbial counts and amine levels.

Taylor (1983), observed high levels of histamine in smoked mackerel products which have been exposed to high ambient temperatures which accelerates the reaction. Use of properly chilled fresh fish with less contamination during the various stages of handling will result in minimum increase in the histamine levels in Spanish mackerel (Trinidad *et al.*, 1986). Hot smoking preserves the product by reducing the bacterial flora and denaturing the enzymes but is incapable of destroying the bacteria toxin already formed. (Poulter, 1998). Thermal death trials by, Bremer *et al.*, (1998) on *H. alvei* isolated from hot smoked kahawai indicated that hot smoking has the potential to eliminate *H. alvei* from seafood products. A retail survey on the levels of histamine in hot smoked products in New Zealand conducted by Fletcher *et al.*, (1998) showed that samples with high bacterial counts has low histamine content and vice versa. Here there was no consistency between the levels of microbial load and histamine content indicating that the histamine has been formed prior to smoking and histamine developing bacteria have been destroyed during smoking.

2.3. Thermal Processing

2.3.1. General Thermal processing

Thermal processing is a method of preserving food by heating in hermetically sealed containers to eliminate the microbial pathogen at a given temperature and specific time. The first book on canning was published by Appert, where he packed food into wide mouth glass bottles, corked and heated and preserved them. However it was in 1864, Louis Pasteur explained that the heating process killed (or inactivated) the microorganisms which extended the shelf-life of food. Several authors studied the link between thermophilic bacteria and spoilage of canned vegetable (Lopez, 1987). Shortly, Peter Durand took a patent for the use of metal canisters. This initiated the beginning of the canning industry (Holdsworth, 1997). In the early nineteenth century studies on the importance of *Clostridium botulinum* and its role in canned foods was established. Bigelow *et al.*, (1920) classified the food based on pH and developed the first scientifically based method for the calculation of minimum sterilization processes for canned foods. This is method is known as the graphical or general method of process calculation. Ball (1923), developed the mathematical or theoretical method for process calculations. Schultz and Olson (1940) developed a nomographic method for process determinations. Ball and Olson (1957) published the first comprehensive book on heat processing followed by Stumbo's book on thermo bacteriology. The mathematical methods which eliminated certain relatively small errors inherent to some of the previous mathematical procedures were developed by Hayakawa and Ball, (1968). In the last 30 years, in addition to Ball, Stumbo, and Hayakawa, Teixeira *et al.*, (1969); Griffin (1969a, b) ; Manson *et al.*, (1970); Manson (1992); Pflug, (1964); Tung and Garland, (1978) and others have further refined mathematical heat process determination concepts and applications. Comprehensive information on all aspects of food canning is available from the research work of Ball and Olson (1957); Lock (1969); Kramer and Twigg, (1970); Pillsbury,(1973); Stumbo, (1973); Desrosier and Desrosier , (1978); Jackson and Shinn, (1979); Hersom and Hulland, (1980); Gilbert *et al.*, (1982); Stumbo *et al.*, (1983); Herbert and Bettison, (1987); Lopez, (1987); Teixiera, (1992) and Larousse and Brown, (1997).

2.3.2. Principles of thermal processing

Thermal process regimes like pasteurization and sterilization vary in the severity of the heat treatment and the purpose of the process (Lund, 1975). Pasteurization involves application of mild heat to high acid food (pH <4.5) to inactivate the enzymes and destroying the spoilage vegetative microorganisms present. In Sterilization all pathogenic and most spoilage causing microorganisms in a hermetically sealed container are destroyed and an environment is created inside the package that does not support the growth of spoilage-type microorganisms and their spores. Thermal destruction of bacteria takes place following a first order-semi logarithmic reduction rate. Theoretically a sterile product cannot be produced however long the product is subjected to heating (Fellows, 1990). To determine the extent of heat treatment following factors given in below must be known (Awuah *et al.*, 2007).

a) The type and the heat resistance of the target microorganism, spore, or enzyme present in the food.
b) The pH, water activity and salt content of the food.
c) The thermo-physical properties of the food and container shape and size
d) The heating conditions.
e) The storage conditions following the process.

Classification of foods based on pH (Ramaswamy & Abdelrahim, 1991) is given in Table 4. Acid foods have a natural pH of 4.5 or below. Low acid foods have a pH greater than 4.6 and a water activity above 0.85. It is scientifically proven that *Clostridium botulinum* does not grow and produce toxin below a pH 4.6. (Gavin & Weddig, 1995). Hence the pH of 4.5 is kept as the demarcation line between high and low acid foods. In low acid canned foods there is every chance that *C.botulinum* may survive if under processed or not adequately stored. These are anaerobic rod shaped bacteria capable of producing toxin if the spores are allowed to germinate.

Table 4. Classification of Foods Based on pH (Ramaswamy & Abdelrahim, 1991)

pH Class	Typical Foods
High acid pH<3.7	Fruit juices, cranberry sauce, fruit jellies, , grape fruit pulp, , orange juice, plum., sour pickles, sauerkraut, vinegar.
Acid pH 3.7- 4.5 pH	Fruit jams, fruit cocktail, Grapes, tomato, peaches, pineapple slices, potato salad, prune juice, vegetable juice.
Low acid pH>4.6	All meats, fish, vegetables. mixed entrees (beans and pork, chicken with noodles, etc.) and most soups

2.3.3. Microbial Destruction Kinetics

Microorganisms differ in their characteristics and thermal resistance. This resistance depends on endogenous factors like genetic, age, moisture content, and competing microbial flora and on exogenous factors like presence of lipids, protein stabilizers, organic salts etc. Spore forming bacteria are of main concern in low acid foods especially when the vegetative growth has been restricted. Bacteria like Clostridium and Bacillus produce endospores that contain essential cellular components and show no metabolic activity for prolonged survival under adverse conditions. In canned foods the primary area of concern is the prevention of the germination and growth of the surviving spores. The minimal thermal process concept introduced by USFDA in 1977 is defined as the application of heat to food, either before or after sealing in a hermetically sealed container, for a period of time and at temperature scientifically determined to be adequate to ensure the microorganisms of food spoilage and pathogens are eliminated or inactivated. Stumbo (1973) summarized various factors that influence the thermal resistances of bacteria and also conditions present during sporulation (temperature, ionic environment, organic compounds, lipids, age, or phase or growth) and conditions present during heat treatment (pH and buffer components, ionic environment, water activity, and composition of the medium). *C. botulinum* is the microorganism of public health concern in low-acid canned foods, due to its high thermal resistance and its capability of producing spores. *C. botulinum* can produce super dormant spores with high thermal resistance (Sebald, 1982). Low pH or acidification reduces the thermal resistance

depending upon the nature of acidifying agent used (Lynch & Potter, 1988). Long chain fatty acid and presence of calcium and iron in the medium also increased the thermal resistance of spores.

Stumbo (1973), have shown various procedures for experimental evaluation of thermal destruction kinetics of microorganisms. The thermal destruction rate of the test microorganism must be determined under the conditions that normally prevail in the container so that an appropriate heating time can be determined at a given temperature.

2.3.4. Survivor Curves and D-value

Thermal destruction of microorganisms follows a first-order reaction indicating a logarithmic order of death (Esty & Meyer, 1922). The logarithm of the surviving number of microorganisms following a heat treatment at a particular temperature plotted against heating time will give a straight line. These lines are commonly called survivor curves. The microbial destruction rate is defined as a decimal reduction time (D-value), which is the heating time in minutes for a given temperature to bring about one decimal reduction in the surviving microbial population. Graphically, this represents the time range between which the survival curve passes through one logarithmic cycle (Ramaswamy & Marcotte, 2006).

2.3.5. Thermal Death Time (TDT) and D-Value

Thermal death time (TDT), is the heating time required to cause death or destruction by subjecting microbial population to a series of heat treatments at a given temperature and testing for survivors. The death in this instance generally indicates the failure of a given microbial population after the heat treatment, to show a positive growth in the subculture media. Comparing TDT approach with the decimal reduction approach, it can easily be recognized that TDT value depends on the initial microbial load (while D value is not). TDT is always measured with reference to a standard initial load or load reduction and represent a multiple of the D-value. For example, if TDT represented the time to reduce the population from 10^{12} to 1 then TDT is a measure of 12 D values. Deviations of

the logarithmic order of microbial death has been provided (Stumbo, 1973) showing typical survivor curves for each situation: (1) heat activation for spore germination, (2) mixed flora, (3) clumped cells, (4) flocculation during heating, (5) nature of the subculture medium, and (6) anaerobiosis.

2.3.6. Temperature Dependence and Z-Value.

The decimal reduction or D value is a function of the thermal treatment at a given temperature. D value has a linear relationship with temperature and changes inversely. The temperature sensitivity of D-values at various temperatures is normally expressed as a thermal resistance curve with log D-values plotted against temperature. The temperature sensitivity indicator is defined as a z value, which represents a temperature range that results in a 10-fold change in D-values, or graphically it represents the temperature range through which the D-value curve passes through one logarithmic cycle (Ramaswamy & Marcotte, 2006) or how many degrees the temperature has to be raised to shorten the heating time by 90% or in other words to make the destructive effort tenfold (Eistner, 1988).

2.3.7. Heat penetration and thermal process evaluation

The process evaluation and heat penetration of thermal processed products in containers have been researched extensively (Stumbo 1973; Lopez, 1981 and NFPA, 1982). Mathematic modeling has been reviewed by (Hayakawa, 1977 and Holdsworth, 1985). The general method by Bigelow *et al.*, (1920) is also known as graphical trial and error method. Improvements in this method have been done by Schultz and Olson (1940). The numerical method normally uses the trapezoidal rule or Simpsor's rule to calculate the area of irregular geometric figures (Holdsworth, 1997). Formulae methods for calculating the heat penetration makes use of theoretical and empherical formula. Hayakawa (1977 b) first made use of analytical or numerical solutions of theoretical heat equations while the formula method is based on heat penetration data. However Pham (1987), pointed out that the formula methods are somewhat misnomer since they are invariably presented as tables rather than equations. Hayakawa (1978),

further divided formula methods into two groups. First group comprised of methods that calculate the lethality at the cold spot for example such as that of Ball (1923); Jakobsen (1954); and Ball and Olson, (1957). Second group consists of methods that calculate mass average lethality for whole containers. Such methods have been developed by Gillespy (1951); Ball and Olson (1957); Stumbo (1973); Hayakawa (1969) and Jen *et al.* (1971). This grouping is similar to what is in some places referred as biological method (Biological Indicator Units) or thermocouple method commonly used to estimate process lethality.

Time-Temperature data for heating and cooling are collected by using thermocouples which are inserted into the geometric centre or cold spot determined for the container. Thermocouple output is measured using a data recorder. The heat penetration parameters are determined by plotting temperature deficit (Tr-T) on semi log paper (temperature difference on log scale and time on linear scale). The intercept is obtained by extending the straight line portion of the curve to the Y axis representing pseudo initial temperature (T_{pih}). The lag factor for heating (Jh), slope of the heating curve (fh), time in minutes for sterilisation at retort temperature (U) and lag factor for cooling (Jc), fh/U, final temperature deficit g, process time B and total process time (T_B) are calculated by the mathematical method of (Stumbo 1973). Total process time was determined by adding process time (B) to the effectiveness of the come up time which has been established to be 58 %.

2.3.8. F-value

F-value is defined as the number of minutes at a specific temperature required to destroy a specific number of organisms having a specific z value (Potter & Hotchkiss, 1995). For convenience, this is defined as an equivalent heating of 1 min at a reference temperature, which is usually taken to be 121°C for the sterilization processes. Thus the F value would represent a certain multiple or fraction of the D-value depending on the type of the microorganism. Time-temperature combinations are used by processors to integrate the lethal effects of microorganisms. The combined lethality so obtained for a process is called process lethality and is also represented by the symbol Fo. From microbio-

logical safety point of view, the assurance of a minimal lethality at the thermal center is of utmost importance, while from a quality standpoint it is desirable to minimize the overall destruction throughout the container.

The minimum process should be severe enough to reduce the population of *C. botulinum* through 12 decimal reductions. Based on published information, a decimal reduction time of 0.21 min at 121°C (Stumbo, 1973) is normally assumed for *C. botulinum*. A 12-decimal reduction would thus be equivalent to a Fo-value of 12 x 0.21 = 2.52 min. The minimal process lethality (Fo) required is, therefore, 2.52 min.

2.3.9. Cook value

Cook value (Cg), a measure of heat treatment with respect to nutrient degradation and textural changes that occur during processing, is determined by measuring the extent of cooking and nutritional loss during processing in a manner similar to the D value, except that the reference temperature is 100° C instead of 121° C, and the z value is 33° C, which is required for the denaturation of thiamine (Ranganna, 2000). Theoretical evaluation of quality changes during thermal processing has been expressed as cook value (Ohlsson, 1980 b)

2.3.10. Factors affecting heat penetration

Penetration of heat into the food is influenced by several factors and a clear understanding is necessary to obtain good results in commercial operations. The characteristics of the retort, the container used, heating medium, filling medium, the temperature gradient between the container and retort, ratio of liquids to solids in the pouch contents, arrangement of containers inside the retort, steam distribution etc. are some of the important factors to be taken care of (Balachandran, 2002). Duckwall (1905), studied the rate of heat penetration in various foods. Zavalla (1916), studied the effect of filling media and the effect of air in steam retorts and the advantage of jumble stacking of cans in the retort for obtaining better heat penetration. Ingredient related factors also affect heat penetration in cans, where fatty tissues are poor conductors of heat. Solids with

gelling properties also absorb water and change solid liquid ratio thereby affecting heat transfer. Liquid and semi-liquid foods are mainly heated by convection while solid foods are heated by conduction. In semi-liquid products heating is by both convection and conduction implying a longer process time due to the slow rate of heat transfer (Clifcorn *et al.*, 1950). Rotation of the cage of the retort during heating significantly increases the rate of heat penetration. (Bindu & Gopal, 2008: Ali *et al.*, 2006). Shape and size of the container affects the heat penetration because smaller containers heat more rapidly due to the larger surface area in relation to the volume of the container.

2.3.11. Heat Sterilization process in retort pouches

Retort pouch process filling is similar to can filling and requires the same care and attention. The major steps in retort pouch packaging are filling, air removal, sealing, traying, autoclaving and cooling (Madhwaraj *et al.*, 1992). Once the product is filled and sealed it is then subjected to temperatures of 121.1°C with counter pressure so that the cold point or slowest heating point within the food reaches the predetermined time temperature integral (Brody, 2003). Once this temperature is reached, the product is cooled, labeled and stored (Mardhwaraj *et al.*,1992; Balachandran, 2002 and Venugopal & Shahidi, 1998).

There are mainly two types of retort pouches viz, preformed and pouches which are made from laminates on the process line. Preformed retort pouches are more commonly used and they are filled manually or by using automatic filling machines. Sauces and curry products are packed instantaneously in pouches that are produced from laminated rolls which are simultaneously formed, filled and sealed (Yamaguchi, 1990). In case of products with solid contents, either pouch are filled with solids together with some liquid and sealed using a vacuum sealing machine.

Extreme care should be taken during filling of pouches so that there is no contamination of the seal area, since this would result in improper sealing. Duxbury *et al.*, (1970) reported that there should not be filling within 1.5 inches of the open top of the package so as to minimize the product contamination to the seal area. Nughes, (1971) suggests leaving as much as one third of the pouch

volume free for the same reason. Lampi and Rubinate, (1973) reported that a significant percentage of process related failures was due to the contamination of seal areas during the filling operation. Schulz and Mansur, (1969) indicated that steam flushing not only cleaned seal surfaces but also removed residual air from the pouch.

Residual air inside the pouch will affect the heat transfer, product quality and seal integrity .of the pouch. The residual air in the pack should be less than 2% of the volume of the pouch contents (Venugopal, 2006) and higher levels of air in the pouch may result in deflating of the pouches during thermal processing. The most commonly used methods for removal of residual air is vacummisation and steam flushing. Vacuum chamber (Goglio, 1968), counter pressure (Tsutsumi, 1972), steam flush (Schulz & Mansur, 1969), and water head pressure (Heid, 1970) are some of the air removal techniques. Air from the solid pack pouch can be removed with the help of vacuum machine and in semi-solid type by the help of steam injection method. Super heated steam is generally used because it causes less moisture condensation in the seal area. Stretch method is applied effectively for the curry types of products (Tsutsumi, 1972). For large pouches, vacuum-sealing machine is very effective to remove the residual air (Yamaguchi *et al.*,1972). Residual air affected the physicochemical, sensory properties and shelf life of wet pack pears (Olives, 2002).

Sealing is an important stage in the operations for retort pouch packaging. Methods of sealing flexible polymeric film pouches have been reviewed thoroughly (Young, 1975 and Brown & Keegan, 1973) and the equipment aspects by McGillan & Neacy, (1964) and McCloskey, (1971). A seal width of 5-10 mm is desirable for good seal strength. Hot-bar sealer and the impulse sealer are commonly used for retort pouches (Tsutsumi, 1974, 1975). Hot bar sealing method is more preferable than impulses sealing since in latter the seals are narrower. Hence, the pouches should be double sealed to reduce the risk of seal defect (Nieboer, 1973). It has been reported that the overseal of retort pouch should be extended over the mouth of the pouches to prevent mold growth in any package above the closer seal (Venugopal, 2006).

Sterilization is usually done in a batch or continuous retort systems. The filled pouches are laid on trays or racks to maximize uniform heat transfer. An additional mesh restraint over the trays is used to restrict pouch inflation and distortion in the retort (Jeffs, 1984). The temperature and duration of the process depends on a variety of factors like type and size of the product and container, type of retort, types of heating medium, etc (Ramaswamy & Singh, 1997). Usually the product is retorted at 121.1°C for a predetermined time. Retort pouches have the tendency to burst open due to the development of internal pressure developed by expansion of headspace gases during retorting. Over pressure is supplied to the retort to counter the steam pressure developed during heating and cooling (Bhowmik & Tandon, 1987 and Tung *et al.*, 1990).

Different types of retort systems and their operations are thoroughly described by different authors (Lampi, 1977; Yamaguchi, 1990, Venugopal, 2006). Steam air mixture and water immersion over pressure retort are commonly used for thermal processing of food in retort pouches. Pflug, (1964) and Pflug and Borrero, (1967), using both laboratory and commercial batch retorts made a comparative study of steam, steam-air mixtures, and water as processing media. In continuous retorts hydro lock sterilizer was used for processing pouches (Lawler, 1967 and Goldfarb, 1970).

After retorting the pouches are removed carefully from the retort and washed in chlorinated water to avoid post process contamination and dried using air knives to remove the water and packed in suitable cartons to facilitate display on shelves of supermarkets and further transportation.

2.3.12. Effect of rotation on heat penetration characteristics

Several factors like retort temperature, product viscosity, head space and rotation speed, rotary diameter etc.will affect the heat penetration rate into the food particles sterilized in a retortable pouch or can (Ghani, *et al.*, 1999 and Krishnamurthy, *et al.*, 2001). Pflug and Barrero, (1967) observed that heat transfer coefficient was one of the critical processing factors. The rate of heat transfer depends on the circulating rate of heating medium across the pouch surface (Peterson & Adams, 1983). Rotation or agitation will help in faster heat

penetration and a quicker attainment of the recommended Fo value. Rotation is more applicable when a filling medium like oil or brine is used. This is mainly because the contents gets agitated during rotation thereby eliminating cold points and bring in more contact to the product. Moreover the heating will be faster and a shorter process time is achieved thereby giving a product with better sensory and nutritional qualities and reduced nutrient losses (Smout, *et al.,* 2000). Thermal softening of the texture of vegetables due to agitation has been reported by Taheran and Ramaswamy, (1996). Excessive heating produces losses in the nutritional quality and organoleptic properties of foods (Hayakawa & Timbers, 1977). Ramaswamy and Sablani, (1997a and b) also recorded the effect of particle shape and particle motion on heat transfer in cans during end over end rotation and the influence of rotational speeds. Increase in rotational speed (rpm) in an end over end rotation or axial rotation has resulted in an increase in heat penetration rate in liquid and semi liquid foods, (Ansar *et al.,* 2006; Berry *et al.*, 1979; Berry & Bradshaw, 1980, 1982; Naveh & Kopelman, 1980; Berry & Dickerson, 1981 and Berry & Kohnhorst, 1985). Vanloey *et al.,* (1994) have found that increasing the rate of rotation is limited, since at higher rotational speed of 20 rpm there is breakage of the product. At high rotational speeds the centrifugal force becomes more than gravitational force resulting in no or poor mixing of the product in the pouch and hence the heat penetration can be slower.

2.3.13. Nutritional quality of thermal processed products

Processed foods should be either refrigerated or heated at high temperatures to eliminate pathogens and microorganisms. Although some of these changes are desirable, prolonged heating at high temperatures would result in unwanted chemical reactions, resulting in loss of nutrients and sensory qualities. Canning is an important method of preservation of fish (Aitken & Connell, 1979). The flesh of certain fishes cannot be canned because they disintegrates after heating and hence commonly canned species are tunas and bonitos , sardines, herring, shrimps and prawns and salmon. The process should be designed in such a way that nutritional constituents present in the initial matter are retained to the maximum to serve human nutrition (Aubourg, 2001). Severe

heat treatment permanently destroys the spoilage bacteria, deactivates enzymes, proteins and vitamins.

Heat processing or sterilization is the most drastic step carried out during the manufacture of canned products and by definition guarantees the sterility of the product (Aubourg, 2001). To keep the quality of the canned fish, three conditions have to be maintained. Firstly the container should be hermetically sealed and the seal integrity should be guaranteed so that the can is sterile all the time (Lopez 1987). Secondly, adequate thermal process lethality to kill the target organism should be given. The temperature at the cold spot which is the most inaccessible part of the food should be recorded by heat penetration studies (Banga *et al.*, 1991). Time and temperature studies depend on the characteristics of the product and container, geometry of the package and the type of heating medium (Lund, 1975, Oliviera *et al.*, 1986 and Vietes *et al.*, 1997). Finally a scrupulous and hygienic post process treatment should be carried out and the products should be stored adequately. The water used for cooling should always be chlorinated so that it is not a source of contamination. Kramer, (1982) and Ruiz-Roso *et al.*, (1998) suggested 3-4 months canned storage to obtain advantageous textural changes and optimal palatability in most canned fish products. Thermal processed products should be stored at ambient temperature much below 30°C in order to prevent the outgrowth of thermophilic spores which may have survived the processing. The effect of storage temperature and duration of storage also is very important for fish products preserved in sauces which are acidic in nature and have corrosive action on the containers used (Lopez, 1987).

2.3.14. Canning of tuna

Canned tuna are considered highly nutritious because of the high omega - 3 polyunsaturated fatty acids (PUFA) content. (Medina *et al.*, 1995a and Gallardo *et al.*, 1989). PUFA is considered to be beneficial to human health for the control of cardiovascular diseases (Carroll & Braden, 1986). The raw material composition, process condition and filling medium have a great effect on the lipid composition (Perez- Camino *et al.*, 1991 and Hale & Brown, 1983). Filling media

may result in differences in the heat penetration and may even extract some components from the fish muscle (Aubourg *et al.*, 1990). The two most commonly used filling medium in the canning industry are brine and oil. Different types of vegetable oils are used. Virgin olive oil is considered to contain natural polyphenols having a role in oxidation (Papadopoulos & Boskou, 1991). Tuna protein has high nutritional value and Duel *et al.*, (1946) reported that tuna protein yielded higher biological values than casein. Pigott and Tucker, (1990) after studying the essential amino acids found that the composition of tuna protein was of very high quality. Heat processing and storage of the canned product can facilitate amino acids, vitamins and minerals to leach out into the medium, leading to significant loss of nutrients if not consumed along with the fish solids. (Aubourg *et al.*,2001). During canning proteins get denatured due to the excessive heating and releases water to the medium. Proteins, minerals and vitamins get released and give a curdled appearance to the contents on opening the pack. Curd formation is noticed in some of the canned fish products like mackerel and salmon. Curd is gelatinous off-white substance that forms on the surface of canned fish. This is due to the heat coagulation of soluble proteins that have exuded from the cut surface of the fish during thermal processing (Tanikawa *et al.*, 1952). Fish with higher oil content tend to give a less curdled appearance due to the effect of lipids on water migration (Aubourg *et al.*, 2001).

2.3.15. Biochemical Parameters

2.3.15.1. Proximate composition

The moisture content of tuna decreases during steam cooking (Castrillion *et al.*, 1996) and the same trend has been observed for sardines (Puga & Diaz, 1989). Canning has found to decrease water content in albacore tuna and increase the protein and fat content (Garcia - Arias *et al.*, (1994). In the case of lipids there was an increase in the fish muscle after canning in oil medium. The lipid content of various fishes were studied by several authors (Gallardo *et al.*, 1989: Hearn *et al.*, (1987). Changes in the fat content and protein content during the canning of fish has been studied by Palle's *et al.*, (1985), Aubourg *et al.*, (1990) and Garcia - Arias *et al.*, (1994) and follow a similar trend. Mai *et al.*, (1978) reported that during cooking, food may lose or gain weight by dilution of

components or by absorption from the filling medium. Longer sterilization time increases ash content due to the absorption of salt added in the filling medium. Increase in fat and ash content decreased the protein in the canned product, with a higher decrease for longer sterilization (Castrillion *et al.,* 1996).

2.3.15. 2. Amino acid profile

Thermal processing affects proteins in two ways. On one hand it results in changes in the secondary, tertiary and quaternary structure of proteins which breaks the bonds and unfolds the proteins and improves their bioavailability since peptide bonds become readily acceptable for intake into the human body. On the other hand alterations in the primary structure may lower digestibility and produce proteins that are not biologically available (Swaisgood, 1985). Phenomena resulting in improvement in loss of both nutritional and physiological properties of food proteins result from the protein denaturization and chemical modification of amino acids (Finot, 1997). In the canning processes, the changes in protein occur mainly at three different stages, namely pre-cooking, thermal processing and diffusion into the filling media. Bender, (1972) and Broek (1965) have reported the effect of thermal processing on fish proteins. Seet and Brown (1983) reported no increase in amino acid content of cooked vs canned tuna. Comparison of thermal processed and raw materials have shown that there is a significant loss in cysteine content. Geiger and Borgstorm, (1983) found that the nutritive value or amino acid content of fish is not destroyed during careful processing. Fellows (1990) reported a reduction of about 10-20% of amino acid in canned foods. Lou (1997) reported a decrease in purine content of shrimp during thermal processing. Severe heating at high temperatures brings about changes in the loss of amino acids like Lysine, L- arginine and L- histidine (Awuah *et al.,* 2007). The loss of lysine is important to the diet since it is an essential amino acid. Lysine due to its highly reactive amino group, is the most chemically modified amino acid. Tooley and Lowrie (1974) found about 25 % loss in lysine content during thermal processing. The loss of lysine in fish is less due to its smaller levels (Hurrel & Carpenter, 1977). Seet and Brown (1983) found only small changes in protein digestibility and available lysine in canned albacore processed in a batch retort and flame sterilization. Banga *et al.,* (1992) developed

a kinetic model for thermal degradation of available lysine and protein digestibility for albacore in oil and found no significant changes in the parameters.

2.3.15.3. Fatty acid profile

Marine lipids are an important source of unsaturated fatty acid and as such are of great nutritional significance (Piclet, 1987 and Simopoulos, 1997). Marine lipids contain a high content of unsaturated omega fatty acids which have proven health benefits (Illingworth & Ullmann, 1990). Since the lipids are highly unsaturated loss of quality is likely to occur during processing and storage (Pearson 1977). Shiau and Shue,(1989) reported that frying of Tilapia fillets prior to canning releases moisture from the meat into the oil which hydrolyses triglycerides to form FFA, diglycerides, monoglycerides and glycerol. Gallardo *et al.*, (1989) observed that there is an increase in PUFA and a decrease in the saturated and mono lipid content of precooked albacore. When oil is added as the filling medium, the fatty acid in the fish react with those of the oil and vice versa and alterations occur in the fatty acid content of both the fish and the oil medium (Garcia *et al.,* 1994; Ruiz-Roso *et al.,* 1998). These interactions between the two fatty acids continue till equilibrium is reached throughout the canned storage (Garcia *et al.,* 1994 and Aubourg *et al.*, 1998). Aubourg *et al.*, (1990) reported a decrease in the lipid content of canned and cooked samples. FFA and phospholipids increased significantly during canning. Hale and Brown,(1983) recommended the usage of filling media containing high PUFA content to retain the positive medical benefits of omega 3 –PUFA present in the fish products.

2.3.15.4. Biogenic amines

Biogenic amines particularly histamine is a significant amine even in canned products due to its ill health. Histamine once formed in a product cannot be destroyed by heating and hence fish that has not been refrigerated adequately before thermal processing cannot be made safe for consumption. Luten *et al.,* (1992) have found that majority of the biogenic amines remain as such in the fish muscle after thermal processing and there is no significant change in their

content before and after thermal processing. This is in agreement with the observations of Windyga *et al.*, (1992), Murray *et al.,* (1982) and Hall *et al.,* (1995). The level of histamine in fresh tuna has been used as an indicator of decomposition prior to canning (Mietz & Karmas, 1977). Shalaby, (1990) found that there is a partial decrease in the histamine levels during canning cycle. But this change is so negligible that the fish cannot be used as a material for canning (Arnold & Brown, 1978). This decrease in histamine may be due to the leaching of the amine into the filling medium and hence cannot be taken as a significant reduction in amine levels. Frank *et al.*, (1981) have seen that histamine levels in immediately caught tuna are negligible. Fran and Sims, (1987) found a decrease in the histamine levels in canned tuna subjected to precooking and retorting, but found that higher levels of putrescence and cadaverine in the final fish samples which showed that the initial raw material was a decomposed one. Tuan and Tsai, (1981) found that histamine content is affected by freshness of fish, fish species, chilling methods, transportation and precooking. Mietz and Karmas (1977) established a chemical quality index for canned tuna for estimating the level of decomposition in fresh tuna prior to canning by using the relationship of dansyl derivatives of five amines (histamine, putrescene, cadaverine, spermine and spermidine) extracted from the canned fish. Veciana *et al.,* (1997) used histidine, cadaverine, tyramine, putrescene as indicators in fresh and canned tuna. Precooking and heat processing of canned tuna lowered biogenic amine levels (Frans & Sims,1987). The higher levels of putrescence or cadaverine in the canned tuna indicate that the fish has undergone decomposition in the raw form. Histamine limits also vary with countries. USDA (FDA, 1982) regulations for canned tuna (albacore, skipjack and yellow fin) is 20 mg histamine per 100 g as an indication that the material has been mishandled or the raw material quality has decomposed and a level of 50 mg/100 gm as an indicator of a potential health hazard.

Bacteria capable of decarboxylating amino acids are found in certain species like enterobacteriaceae, clostridium and lactobacillus. The bacteria responsible for the high histamine levels in fish are *Morganella morganii, K. pneumonia and H. alvei* (Wei *et al.*, 1990, Kimata, 1961; Arnold & Brown, 1978). Since the production of histamine is mainly by bacteria like *Morganella morganii*

and *Enterobacter aerogenes* etc. it is possible to inhibit their growth and amine formation by adding any antimicrobials and preservatives. Spices like clove and cinnamon were found effective against biogenic amine formation (Wendakoon & Sakaguchi, 1992). Histamine toxicity levels have been found to increase in the presence of amines like putrescene and cadaverine and hence FDA suggested the possibility of using these biogenic amines with regard to safety in fish evaluation (Taylor & Sumner, 1987) and FDA, 1995).The problems of histamine fish poisoning would be greater in a tropical country like ours where the average ambient temperatures are high and would result in the growth of these bacteria.

2.3.16. Browning in thermal processed products

Heat treatment triggers browning or maillard reactions which are a complex series of reactions between the amino acids and sugars. Compounds involved in the maillard reaction include amino compounds such as free amino acid and volatile amino compounds associated with microbial spoilage (Nakamura *et al.*, 1973) and carbonyl compounds such as reducing sugars, aldehydes and ketones from lipid oxidation (Pokorny *et al.*, 1973). During the initial stages of the reaction colorless compounds are formed and during the later stages brown coloured pigments called melanoides are formed (Whistler & Daniel, 1985). Even though the characterisitic cooked flavour is desirable, in creating the typical cooked flavour there will be a loss in the quality. Maillard reactions can be inhibited by reducing the pH or temperature if the product is in the liquid form or by decreasing moisture to very low levels. The removal of one of the substrates responsible for browning, mainly sugar may also reduce the reaction (Yamaguchi & Kishimoto, 1976). They also studied the relation of retort pouch thickness and temperature to browning and concluded that minimum browning was achieved at 130°C for 20 mm, 135°C for 15 mm and 140°C for 8 mm. During thermal processing, carbonyl compound from oxidized lipid may be solubilised and react with the nitrogenous compound in the fish flesh to form browning compounds (Fujimoto & Kaneda, 1973).

2.3.17. Changes in vitamins and minerals

Vitamin degradation is dependent on agents like oxygen, light, water solubility, pH and can be catalyzed by the chemicals present. Vitamins are easily affected and degraded by high temperature. Fat soluble vitamins like A, D and E and β–carotene, and water soluble vitamin C (ascorbic acid) , vitamin B_1(thiamine B_2 (riboflavin) are heat sensitive vitamins (Ryley & Kajda,1994). Heat labile vitamins like thiamine, riboflavin, niacin, pyridoxine and panthonic acid are the ones which undergo drastic changes during thermal processing (Banga *et al.*, 1993b). The vitamin thiamine is considerably lost during thermal processing (Chia *et al.*, 1983) and vitamins like A and D which is found in abundant are retained (Bender, 1987). Water soluble nutrients leach into the liquid medium, but in general, nutrient retention in canned seafood products is at a acceptable level (Pigott & Tucker, 1990). Braecken, (1962) found vitamin B_1 levels to be similar for both fresh and canned fish.

Some loss in minerals like sodium, potassium, magnesium, phosphorous, copper, iron and calcium has occurred in canned tuna by leaching into the dipping medium (Seet & Brown, 1983). Fishes with higher fat content produce lesser losses in minerals. Major advantages of thermal processed fish are that the bones become soft and can be consumed, thereby providing valuable calcium.

2.3.18. Changes in smoke components

2.3.18.1. Changes in Total Phenols

Phenolic compounds seem to be mainly responsible for the smoky odour and the major phenols present in smoke flavourings are phenol, *p*-cresol and *o*-cresol. The rate of diffusion of the compound into the fish depends on the character of the surface, type of meat and the type of compound deposited, (Stolyhwo & Siroski 2005). Majority of the phenols are deposited on the surface of the fish and depending on the fat content of the fish penetrate inside. In lean fishes more than 50 % of the mass of phenols can penetrate deeper layers (Kurko & Mezenova , 1985). Phenol content in meat of whole gutted and fillets of

smoked mackerel depend on the smoking conditions and the area of tissue exposed to the smoke (Kolodziejska *et al.*, 2002).

2.3.18.2. Changes in Total Carbonyls

Carbonyls are end products or compounds that are formed due to the oxidation of fat present in the product (Semwal & Arya, 2001). Carbonyls are known to influence the flavour of the fish and fishery products. The carbonyl content of several species of fish and shellfishes by different extraction methods were studied by Ammu *et al.*, (1986). Josephson *et al.*, (1984) studied the carbonyl content of several marine and freshwater fish species. Carbonyls were found to increase initially during chill storage but subsequently showed a decrease (Ammu & Devadasan, 1989). This is in agreement with observations of Tokunaga *et al.*, (1982) who reported that fish held in ice showed a decrease in the aldehyde content unlike fish held in iced water where there was no significant change. Carbonyl value of canned tuna packed in variable ratios of filling media was found to be low (Gu *et al.*, 2001). However, browning of packing medium positively correlated with the carbonyl value since the carbonyl compounds solubilised into the packing medium solution after thermal processing .

2.3.18.3. Changes in Poly aromatic hydrocarbons (PAH)

Most of the PAH in smoked fish comes from the wood smoke. In hot and cold smoked fish there is an increase in the PAH content when compared to the raw fish, depending on the smoking parameters (Tilgner & Daun, 1969 and Steinig & Meyer, 1976). Petrun and Rubenchik, (1966) found that electrostatically smoked fish had a lower Benzo (a) pyrene (BaP) content than those smoked in commercial smoke houses. Kannappan *et al.*, (2000) did not find any BaP in commercially smoked sardine, silvercarp, squid or tuna. Zabik *et al.*, (1996) found that lean and fat trout fillets hot smoked was found to contain BaP. The surface layers of the smoke dried bonito were found to contain 20-40 times more BaP than the meat of the deeper layers (Kikugawa *et al.*, 1986). In smoked fish canned in oil, the contamination can be from the oil used as the filling medium. Certain oils have reported to contain up to 50 µg/kg of PAH. (Stolyhwo & Siroski,

2005). This high PAH content in the oil may be due to the extraction process employed for oil extraction from seed (Slayne, 2003). The PAH content in canned smoked sardine showed that the oil contained 5 times more PAH than the fish flesh(Lawrence & Weber, 1984). PAH compounds are photosensitive and get oxidized. Exposure to light brings about further degradation of the compounds. Simko (1991) observed that immediately after smoking the surface contained 10.6 µg BaP /kg which reduced to 1.3 µg /kg after seven days storage.

2.3.19. Commercial sterility tests

Spoilage of heated foods may be due to under processing where the target lethality is not achieved to kill the microorganisms in the product or through post process leakage or contamination during storage. In this case the contents having been effectively sterilized are reinfected by microorganisms through leaks in the sealed container (Put *et al.*, 1972; Anon, 1968 and Jarvis,1940). The surviving microorganisms are likely to be of several kinds and may include vegetative cells (Bultiaux & Beerens, 1955 and Cameroon & Esty 1940). Any survivors of heat treatment by steam under pressure are very heat resistant bacterial spores, usually one or two kinds (Frazier & Westhoff, 1998).

2.3.20. Shelf life studies

2.3.20.1. Thiobarbituric acid value (TBA)

The severe heat treatment and the presence of certain catalysts in the fish muscle favours lipid oxidation and hydrolysis resulting in off flavors and loss of nutrients (Hsieh & Kinsella, 1989 and Harris & Hall, 1994). Canned tuna muscle with brine as the filling medium had higher TBA values indicating a higher rate of oxidation activity for muscle kept in aqueous medium (Medina *et al.*, 1998). The influence of the physical state of the muscle affect the rate of oxidation of oils (Frankel *et al.*, 1996) Oxidation can increase depending on the partitioning of the PUFA in oil – water emulsion. (Coupland, 1996). Unsaturated fatty acids have high surfactant activities and tend to accumulate at the oil- water interface and hence are more susceptible to lipid oxidation (Coupland, 1996). Damage to the

unsaturated fatty acid can lead to primary and secondary lipid oxidation products, which can result in browning (Aubourg, 1999). A large decrease in the Thiobarbituric Acid reactive substance content in Tuna muscle was found after sterilization followed by storage (Medina *et al.*, 1999). Aubourg and Medina, (1997) and Aubourg *et al.*, (1995 a) observed that primary and secondary lipid oxidation detections were not a reliable method for testing the quality differences in canned products. The same decreasing trend has been reported by several other workers in other thermal processed fish products (Mallick *et al.*, 2003, and Manju *et al.*, 2004, Bindu *et al.*, 2004 and Bindu *et al.*, 2007). TBA reactive substances are highly reactive and react with other food components like the amino groups to produce interaction compound with fluorescent properties (Pokorny *et al.,* 1981).

2.3.20.4. Free Fatty Acid (FFA)

Free Fatty acid showed an increasing trend during the sterilization in different muscle zones of albacore (Aubourg *et al.*, 1990). Time Temperature data of canned tuna processed to F_0 value of 7 minutes indicated that treatments with higher temperatures lead to a higher hydrolysis development even if the processing time was of short duration (Aubourg *et al.*, 1997). The filling medium employed, oil or brine was independent of the extent of free fatty acid formation (Medina *et al.*, 1994). Tanaka *et al.*, (1985) observed a remarkable decrease in the FFA value of the canned mackerel in natural pack processed to equal lethalities at different temperatures. Pre-cooking and subsequent removal of exuded liquid greatly increases the level of FFA in the meat (Medina *et al.*, 1995). Tanaka *et al.*, (1985) also found that at lower temperatures of processing there was an increased level of FFA formation due to the longer process time.

2.3.20.3. Texture Profile Analysis

Texture, appearance and flavour are three important components of food acceptability. Texture can be defined as the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetic (Szezesnaik, 2002). Texture is

influenced by intrinsic and extrinsic factors (Barraro *et al.*, 1998; Sigurgisladottir *et al.*, 2000; Mackie, 1993 and Love, 1983). One of the main problems encountered with fish and fish products is that the fish muscle is very heterogeneous and hence measurements are difficult to reproduce. Factors known to affect textural variation in fish flesh are freshness, size, age, season, pH and other environmental factors (Love, 1979). There is no universal testing method for fish (Heia *et al.*, 1997). Periyam (1967) has studied the effects of food texture on either chewing force or chewing pattern and found that the masticatory parameters are influenced by the material properties as well as the size of the food tested. Bourne (1982) concluded that texture is a group of physical properties that derive from the structure of the food. It is under the mechanical or rheological subheading of physical properties and consists of a group of properties. Texture is perceived by touch, mostly in the mouth and the objective measurements of texture are by means of functions of mass, distance and time only.

Instrumentally texture measurements have been divided into three classes like Fundamental tests, Empirical tests and imitative tests. Texture profile analysis (TPA) falls in the imitative test (Szezesnaik, 1963). Two successive compressions from the Texture Profile Analysis (TPA) results in curves from which several textural parameters can be obtained. Two compressions are said to be necessary, if parameters like cohesiveness, elasticity, adhesiveness, chewiness and gumminess are to be measured (Friedman, *et al.*, 1963 and Szezesnaik, 1963). The height of the force peak on the first compression cycle is defined as hardness. Fracturability or brittleness was defined as the force of the significant break in the curve on the first bite. The ratio of the positive force areas under the first and second compressions was defined as cohesiveness. The work necessary to pull the compressing plunger away from the sample is defined as adhesiveness. The distance that the food recovered its height during the time that lapsed between the end of the first bite and the start of the second bite is described as springiness. Gumminess is defined as a product of hardness x cohesiveness. Chewiness is defined as the product of gumminess x springiness. Gumminess and chewiness are mutually exclusive and hence while reporting TPA values one should report

either value only or not both for the same food (Szezesnaik, 1995). Aitken and Connell, (1979) reported that unless supported by sensory texture evaluations, instrumental methods are of limited use and can be used only by processors and researchers for studying the textural change. Karl and Schreiber, (1985) reported an excellent correlation between maximum shear cell force and first bite hardness and structure retention during mastication for canned fish fillets.

2.3.20.4. Colour Profile

The colour of processed food is an important factor from the consumer acceptability perspective. Naturally occurring pigments and components may be degraded or destroyed during heat processing. Carotenoids present in the fish and meat products are isomerised from 5,6-epoxides to 5,8-epoxides which have less colour. Anthocyanin is changed by heat to brown pigments. High-temperature, short- time minimizes the thermal changes considerably and hence has advantages over conventional retorting where the changes are on a larger magnitude (Awuah *et al.*, 2007). Heating denatures myoglobin and oxidizes carotenoid pigments (Haard, 1992). Free riboses are responsible for majority of the maillard type of reaction that occurs when fish is heated (Tarr, 1958). The fish muscles have a lower water activity and hence browning takes place at a faster rate (labuza, 1972). Trout, Pollack and shrimp processed to an equal lethality in cans developed a darker colour than ones processed in retortable pouches; This was attributed to the longer process time in cans (Chia *et al.*, 1983). By measuring the intensity of colour in the liquids of canned sardines processed for a longer time, Tanaka and Taguchi (1985) found that loss of sugars and lysine is more even if the material was heated at a lower temperature for a longer time. Color changes are more pronounced when the raw material is of poor quality. The green colour discoloration in canned tuna is attributed to the TMAO, myoglobin, cysteine concentration and the cooking operation itself (Khayat 1978). Determination of a combined TMAO and TMA content of the raw fish can be used to indicate the probability of greening occurring during the heat process. (Yamagata *et al.*, 1971).

2.3.20.5. Sensory Tests

The most widespread means of evaluating the edibility of the fishes are the senses- smell and sight, supplemented by taste and touch (Farber, 1965). Sensory evaluation is the subjective taste panel that is used as the standard to determine the accuracy of any objective test (Gould & Peters, 1971). Sensory evaluation is still the most reliable method for evaluation of the freshness of raw and processed fishery products. Heating of meat is accompanied by changes in appearance, smell, taste, texture and nutritive value. Flavour development during heating involves the Maillard browning reactions, fatty acid oxidation and the formation of low molecular weight volatile compounds like ammonia and hydrogen sulfide. Jarvis (1952) reported that excessive heating of little tuna produced a toughening of texture. Tanaka *et al.*, (1983) evaluated quality of canned sardine (*Sardinella melnosticta*) as a function of initial quality. Tanaka *et al.*, (1985) observed that mackerels canned at a higher temperature had a tougher texture than the ones processed at a lower temperature.

Materials and Methods

3.0 MATERIALS AND METHODS

3. 1. Materials

The different materials used for this study are Tuna meat, flexible pouches and ingredients like oil and salt.

3.1.1. Retort pouches

Four types of pouches were used in the study. One was opaque with aluminum foil and the other three were see through pouches which were foil free. Of the three see through pouches one pouch was two layered. The details of all four pouches are given in Table 5. and the photographs in plate 1a-1d.

Table 5. Different types of retort pouches used in the study

	Details of Pouch manufactures	Description of layers	Code
	Indigenous retort pouch		
1.	MH Packaging Ltd, Ahmedabad, Gujarat	Polyester/Aluminium foil/ cast polypropylene (Opaque)	INOP
2.	Pradeep Lamination, Pune, Maharasthra	Polyester coated with silicon dioxide/ nylon/cast polypropylene (see through)	INST
3.	MH Packaging Ltd, Ahmedabad, Gujarat	Polyester/ cast polypropylene (See through)	INTL
	Imported retort pouch		
4.	Korean Retort Pouch	Polyester coated with Aluminium oxide /Nylon/ cast polypropylene (See through)	IMST

Plate - 1 (a) Imported see through pouch Plate - 1 (b)Indigenous see through pouch

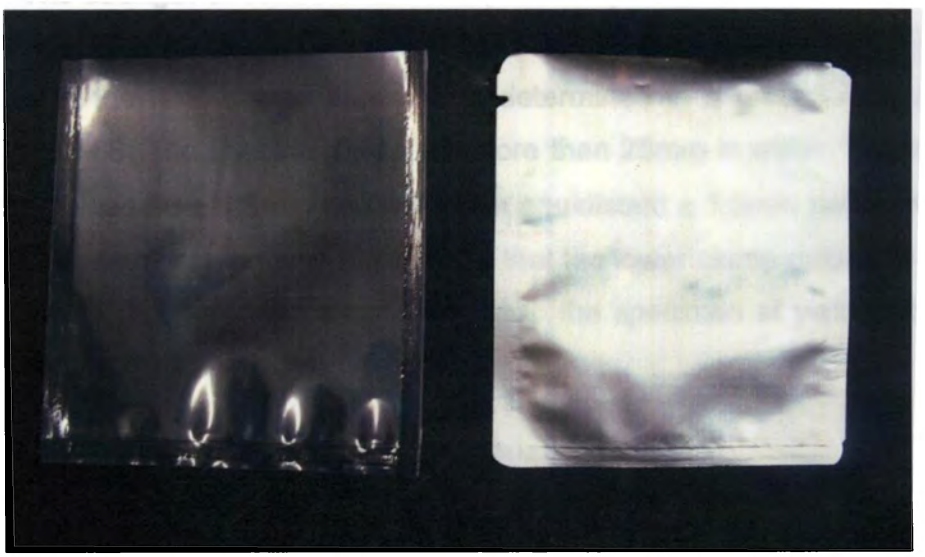


Plate - 1 (c) Indigenous two layered pouch Plate - 1 (d) Indigenous opaque pouch

3.1.1.2. Suitability of retort pouch for thermal processing

3.1.1. 2.1. Thickness of retort pouches

The total thicknesses of pouches were determined as per ASTM (1964).

3.1.1.2.2. Tensile strength and elongation at break (IS: 2508- 1984)

Adequate numbers of strips were cut into suitable size (15 mm x 50 mm). One end of each strip was tightly gripped in the upper clamp after placing the grip loosely in the lower clamp and checking its alignment. The machine was switched on at the pre-adjusted speed (500mm/min). The result of each individual reading to three significant figures in case of tensile strength was recorded. Test strips cut in each principal direction of the paper and tested. The tensile strength at break is calculated in Kg/cm^2 from the original area of cross section. Elongation at break was expressed as % of the original length between the reference lines. The mean values of the 5 results were taken for the calculation.

3.1.1. 2.3. Heat seal strength (ASTM- 1973)

The strength of the seal was determined by measuring the force required to pull apart the pieces of film, which have been sealed together. The breaking strength of the heat sealed seams was determined in a UTM following ASTM (1.73) F.88-68. The specimen was not more than 25mm in width. The initial jaw separation was 50 $\pm$ 1.5mm and the seam equidistant $\pm$ 1.5mm between the two jaws. The rate of loading was adjusted so that the lower clamp moves at a rate of 200mm/min. The maximum stress applied to the specimen at yield or breakage was recorded.

3.1.1. 2.4. Test for Bursting Strength (Duxbury 1970)

The pouch lips were clamped to the burst strength measuring equipment around the air inlet and between the rubber jaws and tightened well. Air was released gradually for 30 seconds. The pouch can hold air for 25 psi pressure

and 30 seconds without bursting and thus it passes the test. It also showed absence of pinholes in the pouch.

3.1.1.2.5. Bond strength (ASTM-1972)

This was measured by initiating the separation of the layers using diethyl ether or chloroform or toluene and measuring the tensile strength.

3.1.1.2.6. Water vapour transmission rate (IS: 1060 Part II -1960)

The test piece was cut using a template, which was of such a diameter that the edge of the test piece covers half the annular recess of the dish. The dish was filled with desiccant to within 1 to 2 mm of the supporting ring. The test piece was placed on the supporting ring and center. The waxing template was placed centrally over the dish and test piece, and molten wax was run into the annular recess until the wax was in level with the top surface of the template. Air bubbles in the wax was broken with a small gas jet, the wax was allowed to harden. The dish was inspected to ensure that the seal is satisfactory and excess wax on the outside was removed. Filling and sealing of the dish was carried out as rapidly as possible so that the desiccant absorbs a minimum of water vapour from the atmosphere. Care was taken not to damage the test area during the operation or to allow the desiccant to come into contact with it. To facilitate the removal of the template from the wax, thin film of petroleum jelly was applied to the beveled edge before sealing and excess wax removed from the lower surface. WVTR was determined by sealing the open end of the dish containing the desiccant (fused Calcium Chloride) by the test specimen and exposing the dish to the desired RH and temperature conditions. Standard test condition was 37°C and 92% RH, when the desiccant used exerts 2% RH. Increase in weight of the desiccant after a known period of time gives the amount of water vapour transmitted by the specimen. The WVTR of the film is calculated as g/ m^2 /24 hrs. at 90 ± 2% RH and 37°C.

$$WVTR = Q \times 24 / A\,t$$

Q - Quantity of water vapour passes through the test material of area 'A' sq.meter for't' hours when the relative humidities on either side maintained at H1 and H2.

Area of test specimen - 50 cm^2.

3.1.1. 2.7. Oxygen transmission rate (OTR)

Oxygen permeability of the film was carried out using gas permeability apparatus (Gas and steam permeability, Ats Faar, Societa' Per Azioni, Milano, Italia) (ASTM, 1982). The test material was cut into suitable size (10 cm dia). 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 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 and D) were closed and the vacuum pump was switched on. Then valve C was opened to create the vacuum in the lower portion and it was checked by tilting central vacuum gauge. It should be preferably 0.2 mm Hg. After that the D valve was opened for purging and a valve for removing the atmosphere gas if any. Then the A and D valves were closed. Mercury (Hg) was transferred into the cell by tilting the outer portion and wait for few minutes to attain 0.2 mm Hg vacuum. Valve A was opened and test gas (O_2) was applied and the pressure was adjusted using the gas cylinder valve. Then timer was turned on and allowed 15 min for stabilization. Initial vacuum reading was noted from the Eurotherm Chassell. At particular interval vacuum was noted and the gas transmission rate was calculated and expressed as mL m^{-2} 24 h^{-1} at 1 atm. pressure at 24°C.

3.1.1. 2.8. Residual air test (Shappee *et al.*, 1972)

The test was performed by holding the pouch inverted below water under a funnel attached to a graduated cylinder filled with water. A corner of the pouch was cut open under the funnel and the air is squeezed out. The amount of residual air in the pouch was measured as the water displacement in the cylinder.

The volumetric measurements of air were corrected to atmospheric pressure by Boyle's law:

$$V_1 = \frac{(P_a - W_h)\ V_m}{P_a}$$

Where, V_1 = residual air in pouch at atmospheric pressure (m L)

P_a = atmospheric pressure (inches of mercury)

V_m = volume of measured air (m L)

W_h = pressure of water in graduated cylinder (inches of mercury)

Where, $W_h = \rho gh$

where, ρ = density of water (kg m^{-3})

g = acceleration due to gravity (m s^{-2} or N kg^{-1})

h = height of water in graduated cylinder (m)

3.1.1.2.9. Overall migration test (IS: 9845- 1998)

Overall migration test was performed by using the food stimulants such as distilled water, and n- heptane. The pouch was filled to capacity with pre-heated stimulant at test temperature and closed. The pouches were exposed to specified temperature and maintained for the specified duration of time (121^0 C for distilled water and 3% acetic acid and 66^0C for n- heptane). After exposure for the specified duration, the pouch was removed and the extractant was quickly transferred into clean glass beaker with three washing with stimulant. The extractant was evaporated to about 50-60 ml and transferred into a clean tarred stainless steel dish along with three washings and further evaporated to dryness in an oven at 100^0 C. The dishes were cooled in a desiccator for 30 minutes and weighed. The extractives were expressed in mg/dm^2.

3.1.1. 2.10. Laminate for product resistance (Gopakumar, 1993)

Two sets of pouches were taken for control and sample. 6 pouches were filled with the product to be packed and other 6 with water (control). All the pouches were sealed. The samples and control were retorted in a pressure retort

suitable for retort pouch processing for 45 and 30 min. for sample and water filled pouches respectively. After processing pouches were cooled to ambient temperature, contents were emptied and washed thoroughly with cold water. The pouches were cut into strips of 1 x 25 mm size from the machine direction and another pouch in transverse direction, cutting across the seam area. The seam was pulled apart and delaminated plies were examined. The laminate plies can be separated apart using Universal Testing Machine and observed the bond strength in g/25mm.

3.1.1. 2.11. Process resistance of pouches (Gopakumar, 1993)

One crumpled and other uncrumpled pouches were placed in a retort containing some water and heated to 121^0 C (15 psi steam pressure). After 30 minutes pouches were cooled and taken out and examined carefully for delamination.

3.1.2. Fishes

Fishes used for the study were yellow fin tuna (*Thunnus albacares*) (Plate 4.) Fishes were obtained from the Cochin fishing harbour, Thoppumpady, Cochin. Fishes were purchased according to the requirement and brought to the laboratory in iced condition. The tuna was washed, bled in chilled water and loined. The red meat was removed from the loins and then the loins were cut into steaks of 1.5 mm thickness (Plates 5 and 6). The steaks were then brined in 5 % brine solution (w/v.) for 1 h. The steaks were then drained and used for the smoking.

3.1.3. Oil

Double refined ground oil was used for filling the pouches for smoked tuna in oil medium

3.1.4. Salt

Salt of edible quality confirming to IS: 594-1962 was used for pretreatment before smoking and for preparation of the filling medium for smoked tuna in brine.

3.2. Machineries and accessories

3.2.1. Kerres Smoke Kiln

The Smoke kiln, Kerres of -Germany (Model No.CS 350 'G' EL) is a stainless unit which has an open chamber. The chamber contains provisions for placing detachable trays on which the fish to be smoked is kept. It has a heating element and fan at the top for temperature control and spreading the smoke in the chamber. There is an inlet for smoke generation below the chamber and an outlet at the top where the smoke escapes out after passing over the fish. Digital controls are provided for setting the temperature and time. Smoke generation is done manually in the chamber below the kiln and the smoke is allowed to pass upwards through an opening in the chamber. The quantity of smoke was controlled by adjusting the inlet valve. There is an inlet to continuously feed the wood once the smoking has commenced. The photograph of the smoke kiln is given in Plate 2.

3.2.2. Pilot scale retorting unit

The pilot scale mill wall model 24 rotary retorting system (John Fraser and Sons Ltd, FWS House, Stoddard Street, New Castle-upon-Tyne, UK) was used for the experiments (Plate 3.). This pilot scale retorting system performs laboratory scale thermal processing in a manner which ensures close simulation with commercial scale equipment and which produces a high degree of process reproducibility and accuracy. This system comprises three major components; the retort, the receiver and the control system. The retort provides a chamber in which the product is subjected to the required thermal process. The receiver provides a pressure to balance the overpressure in the retort during super heated water cooks and during overpressure cooling. The control system provides the means to sequence process events, regulate energy flows and document retort temperature and pressure.

Retort is constructed of mild steel which can withstand a working pressure of 3.5 bars having a dimension of 594 mm inside diameter X 650 mm inside length on parallel portion. It has a standard square cage, which is perforated with

Plate - 2 Smoke Kiln

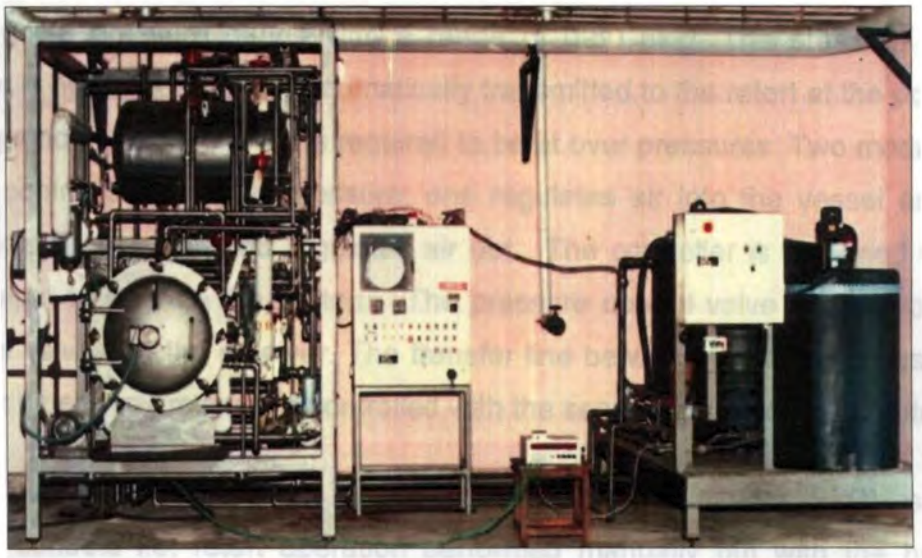


Plate - 3 Overpressure Autoclave

side slots. The speed of rotation of cage ranged from 0 to 51 rpm and was electronically controlled. Instrument pockets are provided on the right side of the shell. These include pressure gauge, retort thermometer, pockets for thermocouple glands and petcock at the rear end. A water gauge is provided on the right hand side of the retort to know the water level inside the retort. A pressure release valve is provided on the retort to release the pressure if it is above 55 psig. The pressure gauge, which is provided on the retort, has got a range of 0 to 60 psig. A 4-blade stainless steel fan is fitted to the retort to create considerable turbulence within the retort during processing to ensure well mixing of steam and that no stagnant air pockets were allowed to exist. The retort is connected to a very efficient cooling system. As soon as the process is over, steam can be switched off and water can be allowed to enter into the retort with the help of a water pump from the water-storing tank. This will provide a very efficient cooling mechanism by spraying water from the top of the retort. The same water can be recirculated with the help of a recirculating pump (Myson MSK 50 – 2/2090).

The receiver is also constructed of mild steel and has got a working pressure of 50 psig having a dimension of the receiver is 594 mm inside diameter X 850 mm on parallel side. It has got a water gauge with the gauge top which indicates the receiver is full and the gauge bottom which indicates receiver below overflow level. It has got a pressure release valve and the setting is on 55 psig. It has got a pressure gauge with a range of 0-60 psig. The pressure in the receiver is hydraulically and pneumatically transmitted to the retort at the points in the sequence when the retort is required to be at over pressures. Two modulating valves control the receiver pressure; one regulates air into the vessel and the other acts on the vent and regulates air out. The controller is designed with a dual output to operate the system. The pressure control valve is connected to the vent valves on the receiver. The transfer line between the two vessels must be open when the pressure is controlled with the sensor mounted on the retort.

The control systems has a Programmable Logic Controller (PLC) assisted manual controls i.e. retort operation performed manually but with the help of discrete electronic programmable input detector controllers for temperature and

pressure. The control system has got a digital temperature indicator and pressure indicator. A digital three-pin circular chart recorder is fitted to record retort temperature and pressure and receiver pressure. A eurotherm digital indicator is fitted to display cage rotation speed. The instrument is connected to the 0-10 V output of the motor control unit and is scaled for 0-51 rpm. A digital electronic timer is provided to assist the timing of the cook period. The timer is integrated into the PLC (Mitsubishi FI series 60 I/O) monitor system and is used to prompt the operator to begin cooling. The PLC system is provided to monitor system safety. It observes retort door interlocks and temperature and pressure alarms and acts upon the automatic valves pump and cage drive (Plate 3).

3.2.3. Packing glands and accessories

Ellab GKM-13009-CXXX packing glands for all kinds of containers were used for the experiments. The GKM is as standard delivered with a GKM-U rubber O ring. For special applications it can be used with wedge washers and silicon washers. Packing glands are usually made up of brass, stainless steel or polyoxymethylene.

3.2.4. Standard thermocouple probes

The probes used for the experiments are that of Ellab Type SSE- G700-SF (ELLAB Co. Denmark) stainless steel electrode with a length of 100 mm and diameter of 1.2 mm. These probes are copper/cupronickel thermocouples; they are sealed probes with the conductor being insulated from the process medium. The pouches are E.M.F. characteristics corresponding to the probe output voltage of Cu/Cu Ni thermocouples.

3.2.5. Ellab CTF 9008 Precision Thermometer and Fo- value computer

Temperature range of the instrument is –100.0 to +350.0°C. Resolution of the instrument is 0.1°C. There are 8 channels with selective functions for product (T_c) and chamber (T_a) temperatures. These 8 channels are updated within 4 seconds with each channel getting updated within 30 seconds. The Fo constants are programmed T=121.1°C, Z=10°C and Cook value constants T=100°C,

Z=33°C. The print out interval from the instrument can be selected and it varies from 30 seconds to 60 minutes. The print out shows Tc and Ta min/max, peak temperatures, channel numbers and the corresponding Fo and cook value of each channel.

3.2.6. Vacuum sealing machine

The vacuum sealing machine (Model QS 400 VD) supplied by M/s Sevana Electrical Appliance Pvt. Ltd., Box No. 2, Kizhakkambalam, Kerala, India, was used for sealing the pouches (Plate 12).

3.2.7. Food Texture Analyzer

It is a general-purpose material-testing machine manufactured by Lloyd instruments, UK (Model LRX plus). The software used in the instrument is Nexygen which gives data output to a computer and printer. The main part of the instrument was fitted with a load cell of 50 N. The LRX plus machine was fitted with two magnetically activating limit stops to stop the machine. The speed of the cross edge movement varies from .01-1016 mm/min. The unit has a liquid crystal display (LCD) to show set up information, load and extension values and a key pad to input information for operating the machine when under the control of the console. The operating status of the machine was shown and described on the display. The display, which has 4 lines of forty characters, was used to show or request information. The lower lines are split into four blocks, one block above each soft key to indicate the function of the key.

3.2.8. Hunter lab MiniScan® XE plus

The Hunter Lab MiniScan® XP Plus spectro colorimeter, model No D/8-S (Hunter Associates Laboratory Inc., Reston, VA, USA) with geometry of diffuse 80 (Sphere-8 mm view) and an illuminant of D 65 optical sensor and100 standard observer was used for instrumental colour measurement of samples. The colour values are expressed using the standard CIE $L^*a^*b^*$ system. L^*, a^*, and b^* values (non dimensional units) refer to the three axes of the system: a lightness axis (white (100)- black (0); L^*) and two axes representing hue and chroma, (a^*) one

red (positive)-green (negative) and the other (*b**) blue-yellow This system provides an unambiguous description of color and has the advantage that color differences between samples can be determined using simple computer programs.

3.2.9. Spectrophotometer

Spectrophotometer of Spectronic 20 Genesys model manufactured by Thermo Spectronic, Rochester, NY 14625 was used for the study.

3.3. Methods

3.3.1. Brining of tuna

The tuna steaks were immersed in 5 % salt solution in the ratio 1: 1 (wt: v) for 1 hour and drained (Plate 7). The drained tuna steaks were packed in laminated covers made of polyester / polythene, sealed and kept overnight in the chilled condition (2 ± 1°C) for equilibration of salt content.

3.3.2. Standardization of smoking parameters

The salted tuna steaks were wiped off excess moisture and surface dried on trays in a smoke kiln at 45 °C for 30 min. Smoke generation was done manually using saw dust from teak *(Tectona grandis)*, *Cheruteak (Callicarpa tomentosa)*, Cashew *(Anacardium occidentale)*, *Kolamavu* (*Buchania axillaries*), Acacia *(Acacia auriculiformis)*, *Maruthu* (*Terminalia paniculata*), *Anjily* (*Artocarpus hirsuta*) and husks from coconut (*Cocos nucifera*). The saw dust from these wood and coconut husks were ignited in different batches and smoke arising from them were allowed to flow over the tuna steaks. The process of smoking is given in Plates 8 and 9 and smoked tuna in Plate 10. The tuna was smoked to different durations of 30, 60 and 90 minutes at 75°C. The quantity of smoke allowed was controlled by adjusting the valve. The steaks were turned over after regular interval to get a uniform colour. After smoking the steaks were cooled and removed from the trays. The selection of wood and duration of smoking was mainly based on the organoleptic characteristics like flavour and colour and the benzo (a) pyrene content of the smoked fish. For preparation of smoked and thermal processed tuna in the three different forms,

Plate - 4 Yellowfin Tuna

Plate - 5 Tuna Loins

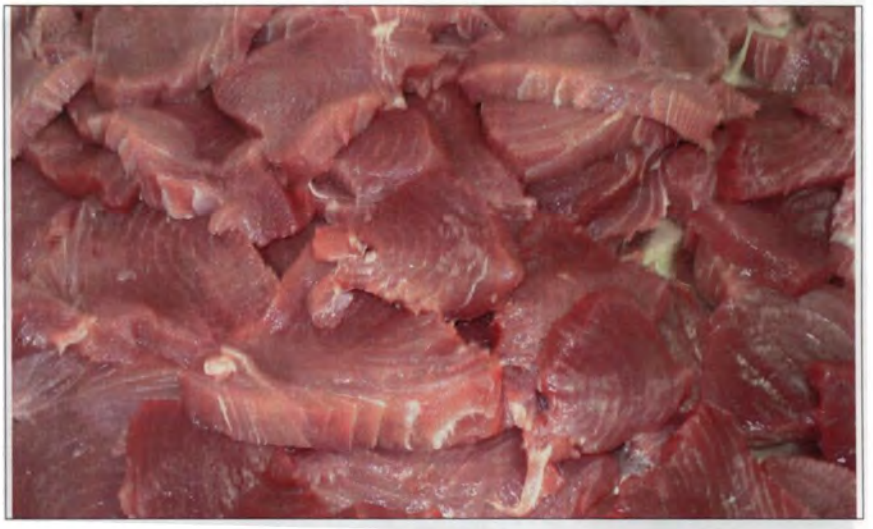


Plate - 6 Tuna Steak

Plate - 7 Brining of Tuna

Plate- 8 Tuna Loaded into Smoke Kiln

Plate - 9 Smoked Tuna in Kiln

Plate - 10 Smoked Tuna

Plate - 11 Weighing and Packing of Tuna

Plate - 12 Vacuum Sealing Machine

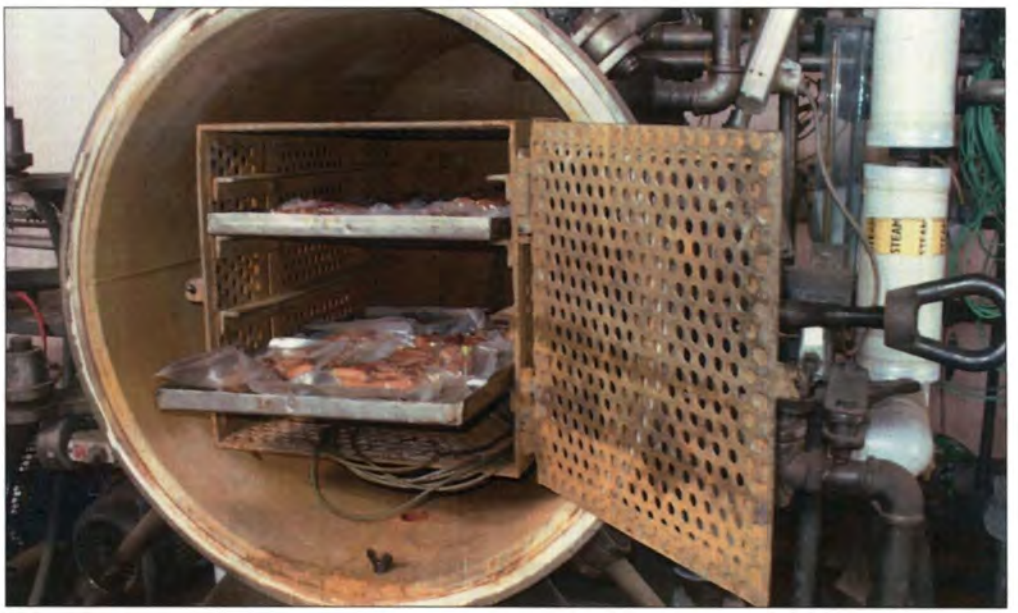


Plate - 13 Loading into Autoclave

Plate - 14 Smoked and Thermal Processed Tuna in Pouches

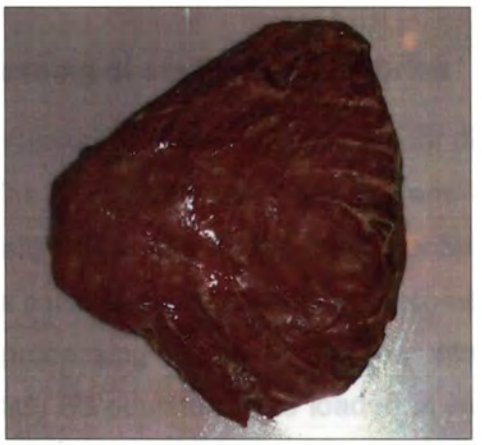


Plate - 15 Smoked and Thermal Processed Tuna Drypack

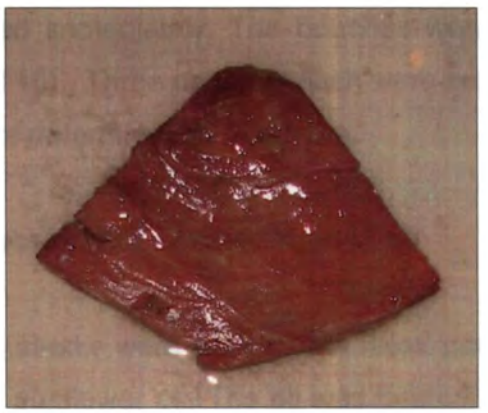


Plate - 16 Smoked and Thermal Processed Tuna in Brine

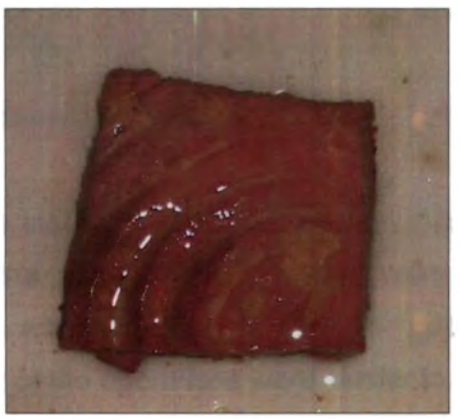


Plate - 17 Smoked and Thermal Processed Tuna in Oil

smoking was done for one hour at 75°C using dried coconut husks, which had a moisture content of about 20-25 %.

3.3.3. Thermal processing of smoked tuna in brine

Smoked tuna steaks were packed into the four different retortable pouches used in the study. The filling ratio was 60 g fish and 40 ml liquid medium. Hot brine solution (1 % salt) was added as the filling medium (Plate 11). The pouches were then sealed in a liquid vacuum packaging machine (Plate 12) and loaded on to trays for further processing in the retort. The retort was loaded to the full capacity. Approximately 50 pouches were loaded in each batch. Pouches were heat processed to a Fo value of 10 min at 121.1°C in a stationary retort (Plate 13). Heat penetration characteristics were recorded using thermocouples connected to Fo value cum cook value recorder. At the end of the process the pouches were cooled immediately. The pouches were then dried, labeled and stored (Plate 14 and 16). Three pouches each were kept at 37°C for 15 days and at 55°C for 5 days for determination of sterility.

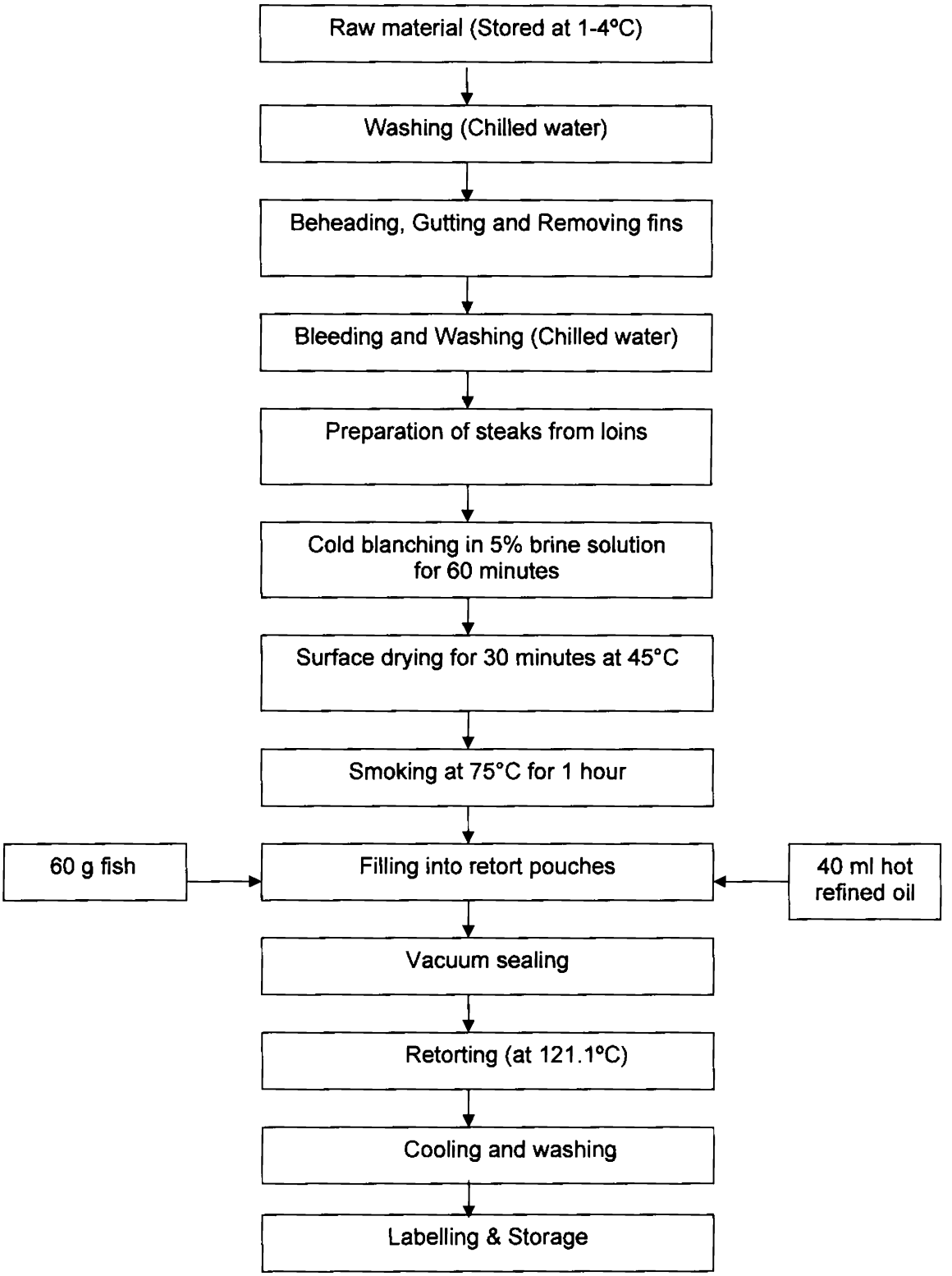
3.3.4. Thermal processing of smoked tuna in oil

Smoked tuna steaks were packed into retort pouches with a filling ratio of 60 g fish and 40 ml sunflower oil. The oil was heated to 90°C and filled into the pouches. All the four pouches were then sealed using a vacuum packaging machine for liquids and loaded on to trays for further processing in the retort (Plate 15). The rest of the operations were similar to as that of tuna in brine.

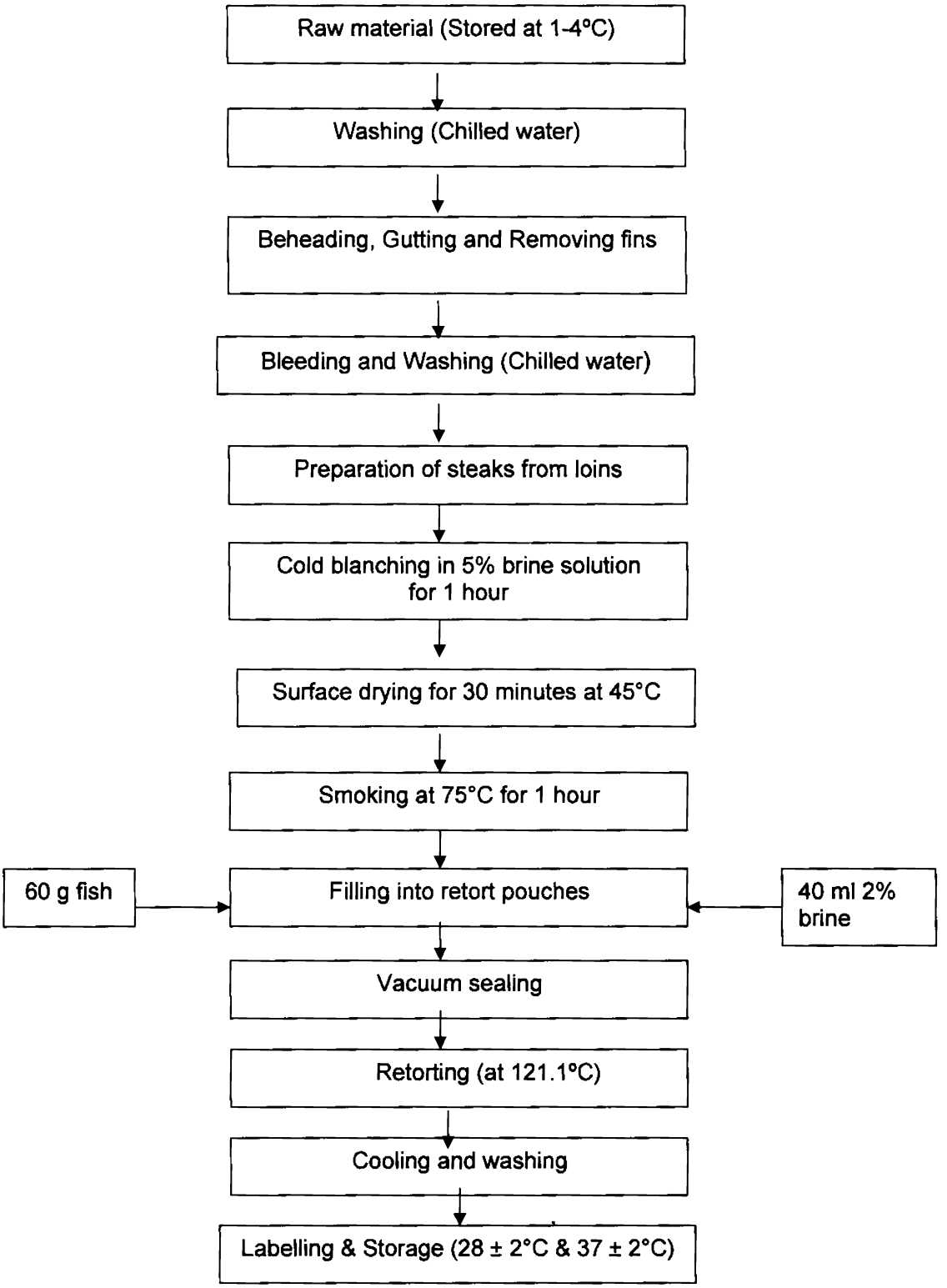
3.3.5. Thermal processing of smoked tuna dry pack

Smoked tuna steaks (100g) were packed into the four different retortable pouches used in the study. The pouches were then sealed in a vacuum packaging machine and loaded on to trays for further processing in the retort (Plate 17). The rest of the operations were similar to as that of tuna in brine.

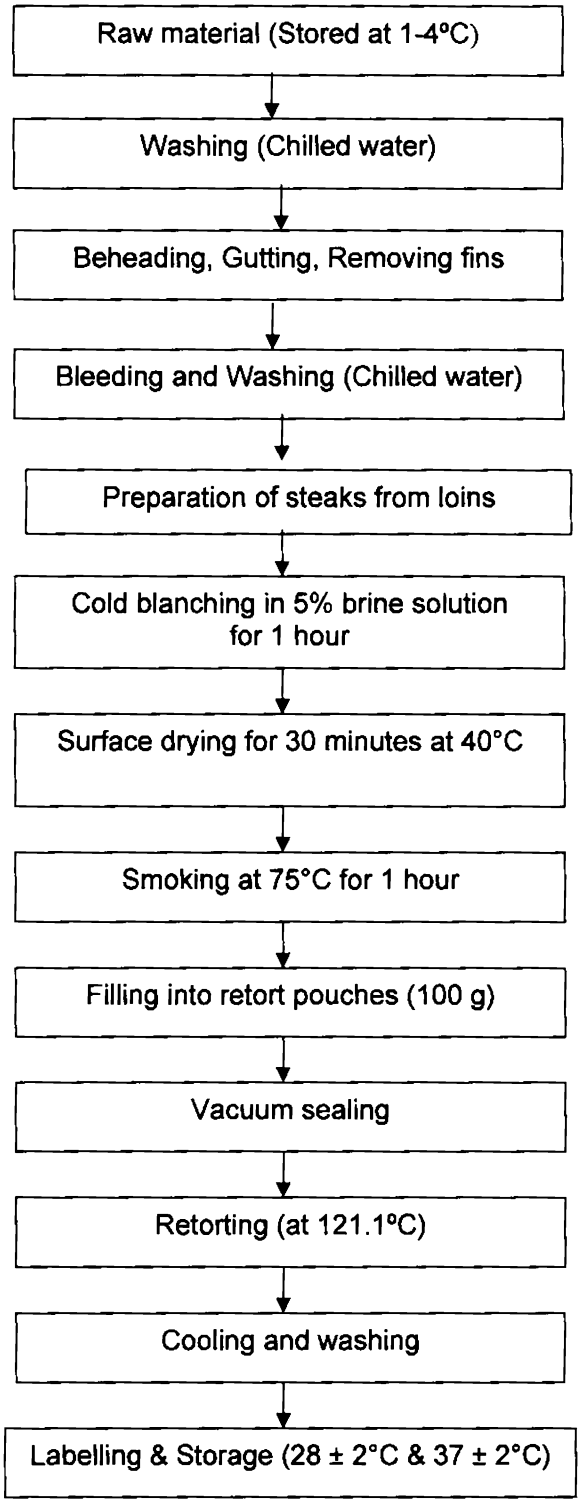
Flow Chart - 1. Pouch packing of Smoked Tuna in Oil



Flow Chart - 2. Pouch packing of Smoked Tuna in Brine



Flow Chart - 3. Pouch packing of Smoked Tuna as dry pack



3.3.6. Heat penetration and thermal process evaluation

The thermal data were taken by inserting thermocouple needles into the product. Thermocouple output was measured by using an Ellab CTF 9008 data recorder. Time-temperature data were taken at an interval of one minute. The heat penetration data were plotted on a semi log paper with temperature deficit (RT-CT) on log scale against time. Lag factor for heating (J_h), slope of the heating curve (f_h), time in minutes for sterilization at retort temperature (U) and lag factor for cooling (Jc) were determined. The process time was calculated by mathematical method (Stumbo, 1973). The graph for Fo value, cook value, retort temperature and product temperature were drawn from the time- temperature data. Actual process time is determined by adding process time (B) and the effective heating period during come up time i.e. 58% of the come up time.

3.3.7. Quality analysis of retort pouch products

Tuna in oil, tuna in brine and tuna as dry pack heat processed to Fo 10 in four different pouches were stored at ambient temperature 28 ± 2°C and also at an accelerated temperature of 37± 2°C to determine the shelf life. Triplicate samples were periodically analysed once in a month for determining changes in thiobarbituric acid (TBA) value, free fatty acid (FFA), pH, changes in texture profile by instrument methods, changes in CIE *L*a*b** colour values and organoleptic sensory methods for determining the overall acceptability.

3.3.7.1. Determination of pH (IS: 2168-1971)

About 5 g of the sample was homogenized with 10 ml distilled water and the pH was recorded using a digital pH meter.

3.3.7.2. Determination of moisture (AOAC, 2000)

A known weight of homogenized sample (10g) was weighed in a preweighed clean petridish on an electronic balance. The samples were allowed to dry by placing in a hot air oven at 105°C for 16 h, then cooled in a desiccator

and weighed until constant weight was obtained. The moisture content was calculated and expressed as percentage.

$$\% \text{ Moisture} = \frac{\text{Loss in weight x 100}}{\text{Weight of the sample}}$$

3.3.7.3. Determination of crude protein (A.O.A.C, 2000)

About 0.5- 1g of the minced sample was transferred into a Kjeldahl flask of 100 ml capacity. A few glass beads and a pinch of digestion mixture (8 parts K_2SO_4 and 1 part $CuSO_4$) and 10 ml of concentrated sulphuric acid were added. It was digested over a burner 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 generated no heat. It was transferred quantitatively into a 100 ml standard flask and made up to the volume. With a 2 ml pipette made up solution was transferred to the reaction chamber of the Micro-Kjeldahl distillation apparatus. 2 drops of phenolphthalein indicator and 40% sodium hydroxide were added till the indicator changed to pink. Distillation was done for 4 minutes and ammonia liberated was absorbed into 2% boric acid containing a drop of Tashiro's indicator. The amount of ammonia liberated was determined by titration with 0.01 N standard sulphuric acid. Crude protein was calculated by multiplying total nitrogen content with conversion factor of 6.25 and expressed as percentage

$$\% \text{ Crude protein} = \text{nitrogen content x } 6.25$$

3.3.7.4. Estimation of crude fat (AOAC, 2000)

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

$$\% \text{ Crude fat} = \frac{\text{Weight of fat x 100}}{\text{Weight of the sample}}$$

3.3.7.5. Determination of ash content (AOAC, 2000)

About 1-2 g of the sample was transferred into a preweighed silica crucible. The samples were then charred by placing in a muffle furnace at 550°C for 4 hrs until a white ash was obtained. Crucibles were weighed after cooling in a desiccator and percentage of ash was calculated.

$$\% \text{ Ash} = \frac{\text{Weight of residue x 100}}{\text{Weight of the sample}}$$

3.3.7.6. Determination of Thiobarbituric acid (TBA) value (Tarladgis *et al.*, 1960)

About 10 g of the sample were macerated with 100 ml 0.2 N HCl and made into a slurry. Slurry was poured to a round bottom flask and connected to a TBA apparatus. The macerated sample was distilled by steam distillation method and 50 ml of the sample was collected in 10 minutes. Accurately weighed 0.288 gms of TBA standard was dissolved in 100ml glacial acetic acid in hot water bath and cooled to room temperature. Five ml of the samples were taken in test tubes and 5ml of the prepared TBA reagent was also added. A blank was also made with distilled water. Then the samples were kept in boiling water bath for 30 minutes for colour development. The developed colour was read at 538 nm wavelengths against blank in a spectrophotometer. The TBA value is expressed as mg malonaldehyde / kg of fish.

3.3.7.7. Determination of Free Fatty Acids (AOCS, 1989)

About 10 g of the sample was blended with anhydrous Na_2SO4 in a mortar. The blend was shaken with chloroform and kept under dark overnight and filtered. 20 ml of the extract was taken in to a clean beaker. Chloroform was evaporated on a water bath and weight of fat was determined. Another 20 ml of extract was transferred in to a conical flask. Chloroform was evaporated off. To this 10 ml of neutral alcohol was added and warmed. It was titrated against 0.01 N NaOH using phenolphthalein as indicator. Percentage of free fatty acid (FFA) was calculated as oleic acid.

3.3.7.8. **Total Volatile Base Nitrogen** (Conway, 1950)

Total volatile base in the sample was determined as total volatile base nitrogen (TVB-N) by the micro diffusion method. One ml of standard N/100 sulphuric acid was taken in the inner chamber of the diffusion unit. To the outer chamber 1 ml of TCA extract was added followed by 1 ml of saturated potassium carbonate. The unit was then sealed with the glass lid and kept undisturbed overnight. The amount of un reacted acid in the inner chamber was determined by titrating against standard N/200 sodium hydroxide with 2 drops of Tashiro's indicator. Similarly a blank was also run. Total volatile base nitrogen (TVB-N) was calculated and expressed in mg N/100g of the sample.

3.3.7.9. **Total Amino acids** (Ishida *et al.*, 1981)

Principle

The amount of each amino acid present within a given protein does not vary from molecule and can provide useful information about the nature of the protein molecule. In a typical analysis of the amino acid content of a protein, peptide bonds are broken by acid hydrolysis with 6N HCl at 110°C (24h) so that the released amino acids can be assayed. The amino acid tryptophan is not stable to acid digestion in the presence of even trace amounts of oxygen and is estimated separately by alkali digestion.

Sample preparation

About 100-150 mg of sample was weighed accurately into a heat sealable test tube. 10 ml of 6 N HCl was added and the tube was heat sealed after filling pure nitrogen gas. Hydrolysis was carried out in an hot air oven at 110^0 C for 24 hours. After the hydrolysis, the contents were removed quantitatively and filtered into a round bottom flask through Whatman filter paper No.42. The contents of the flask were flash evaporated to remove traces of HCl and the process repeated for 2-3 times with added distilled water. The residue was made up to 10 ml with 'C' buffer (sodium citrate tribasic, perchloric acid, n- caprylic acid, pH 2.2).

HPLC Analysis -The sample was prepared and filtered again through a membrane filter of 0.45 μ and 30 μl of this was injected to Shimadzu HPLC-LC10AS consisting of column packed with a strongly acidic cat-ion exchange resin i.e. styrene di-vinyl benzene copolymer with sulfonic group. The column is Na type i.e. ISC-07/S1504 Na with a length of 19 cm and 5 mm diameter. The mobile phase of the system consisted of two buffers, Buffer A (Tri sodiumcitrate-32.7g, Methanol of 140 ml, Perchloric acid 16.6 ml pH 3.2, make up to 2 liter) and buffer B (Tri sodium citrate 117.6 g, Boric acid 24.8 g, 4N NaOH 45 ml, pH 10). The oven temperature was maintained at 60° C. The amino acids were eluted from the column by stepwise elution i.e. acidic amino acids first followed by neutral and then basic amino acids. The amino acid analysis was done with non-switching flow method and fluorescence detection after post-column derivatization with O-phthalaldehyde In the case of proline and hydroxy proline, imino group was converted to amino group with sodium hypochlorite. Amino acid standards (Sigma Chemical Co., St. Louis, USA) were also run to calculate the concentration of amino acids in the sample. Calibration of equipment using standards was done before the start of analysis.

Quantification of amino acids: The standard and the sample were analyzed under identical conditions. The elution time of the amino acids of the sample was compared and identified with those of the standard. Quantification of amino acid was done by comparing the respective peak areas in the chromatogram of the sample and the standard. The amino acid content was calculated as follows,

$$\text{mg amino acid/g tissue} = \frac{\mu\text{mol}*\text{mol.wt}*\text{volume made up}*1000*100}{1000*1000*20*\text{wt. of sample}}$$

The amount of each amino acid is expressed as mg amino acid/ g tissue

3.3.7.10. Estimation of Tryptophan (Sastry and Tummuru, 1985)

About 200-250 mg of sample was hydrolysed with 10 ml of 5% NaOH at 110° C for 24 hours in a sealed tube filled with pure nitrogen. The hydrolysate was neutralized to pH 7.0 with 6 N HCl using phenolphthalein indicator and checked with BDH pH paper. The volume was made up to 100 ml with distilled water. This was then filtered through whatman filter paper No.1 and filtrate was used for estimation. 0.1 ml of 2.5% sucrose and 0.1 ml of 0.6% thioglycolic acid

were added to test tube containing 4 ml of 50% H_2SO_4 and kept for 5 min in water bath at 45-50° C and cooled. An aliquot of the sample was then added to the test tubes. The experiment was repeated with 0.1 to 0.8 ml of standard tryptophan (10 µg/ml). The volume was made up to 5 ml with 0.1 N HCl and allowed to stand for 5 minutes for the development of colour. The absorbance was measured against a reagent blank at 500 nm.

3.3.7.11. Determination of biogenic amines (Ozogul *et al.*, 2002)

The biogenic amines content in fish was determined in HPLC by pre-column derivatisation with benzoyl chloride as described by Redmond and Tseng, (1979) with modification in gradient elution system as per the method of Ozogul *et al.*, (2002) using acetonitrile and water. The gradient system and the flow rate were modified depending on the retention time of the standard amine solution to get good resolution within a short time. The standard amine solution was prepared so as to give a 10mg free base each amine per ml.

Derivatisation of standard amine solution with benzoyl chloride

The benzoyl derivative of the Biogenic amines were done by following the Schotten- Baumann benzoylation reaction under alkaline condition as described by Redmond & Tseng, (1979) but with little modification of the sample. For enhancing the reaction of amines, 2% benzoyl chloride in acetonitrile was used.

For derivatisation of standard amine solution, 50 micro litre of standard amine solution (10mg/ml) was added with 1ml of 2M NaOH followed by addition of 1ml of 2% benzoyl chloride (in acetonitrile). Then it was mixed thoroughly in a vortex mixture for 1 minute. The reaction mixture was left at room temperature (25°C) for 30 minutes to complete the benzoylation reaction. The reaction was stopped by the addition of 2ml of saturated NaCl solution. After that it was extracted with 4ml of di-ethyl ether by centrifugation at 3000 rpm for 5 minutes. Thereafter the upper organic layer was transferred to a clean tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 0.5ml of acetonitrile and 20 µl aliquots were injected for HPLC analysis.

Preparation of sample was done as per the method of (Yen & Hseih, 1991). 10 g of the sample was homogenized with 6% TCA and filtered through

Whatman No.1 filter paper. The filtrate was made up to 100 ml with TCA. 2ml of this extract was derivatised with benzoyl chloride by the same procedure as described in derivatisation of standard amine solution.

Chromatographic condition

Chromatographic separation was done by continuous gradient elution with acetonitrile (solvent A) and HPLC grade Millipore water (solvent B) as described in Ozogul *et al.,* (2002). A gradient started with 50% acetonitrile and increased to 80% in the 6^{th} minute. The pressure was maintained between 48-52 Pascal throughout the separation period. Total separation of seven amines was completed within 9 minutes. Detection was done using Photo Diode Array (PDA) detector.

For calibration curve, 5 standard concentrations of amines mixtures were prepared and injected in a series comprising 10mg/ml, 5mg/ml, 1mg/ml, 0.1mg/ml and 0.01mg/ml standard concentration. The standard curve was prepared corresponding to Hitachi-Merck HPLC System Manager Software. 20 µl sample was injected for analysis.

3.3.7.12. Estimation of Polyaromatic hydrocarbons (Granby and Spliid,1995)

Principle: PAHs, a group of lipophilic compounds are extracted from fish with n -hexane after saponification. The two fractions are eluted in a column of alumina and silica by normal hexane to remove low molecular weight hydrocarbons. On further elution with 1:1 dichloromethance and normal hexane gives high molecular weight hydrocarbons, one of which is benzopyrene, this portion is then dried and taken in mobile phase, acetonitrile and analysed in HPLC.

The method of estimation of PAH compounds was done as per the method of Granby and Spliid (1995) with some modifications. Approximately 10 g of meat homogenate was saponified by refluxing for 2 h with 10 ml of a 4 N KOH solution and 40 ml of ethanol. Then 40 ml of the 4 N KOH solutions were added and the mixture was allowed to stay overnight. Afterward, the sample was transferred to a separatory funnel and extracted with 3 times with 25 ml of n-hexane. Organic phases were dried by filtering through a funnel with anhydrous sodium sulfate and

vacuum evaporated to approximately 1 ml by n-hexane. The chromatographic clean-up was performed on a column (20 cm length, 1 cm internal diameter) filled with 4 g aluminium oxide above 4 g silica gel that had been activated overnight at 250°C and 120°C, respectively. The sample was added to the top of the column and eluted with 25 ml of n-hexane (fraction containing paraffin hydrocarbons) and 30 ml of n-hexane/dichloromethane (1/1) (fraction containing polyaromatic hydrocarbons). This fraction was evaporated to dryness by vacuum and nitrogen flow and dissolved in 1 ml of acetonitrile. The detection of PAHs was performed on a Shimadzu high performance liquid chromatography equipped with photo diode array detector set at 254 nm. The LC column is a Lichrosphor –PAH, RP 18. HPLC is conditioned by passing mobile phase, HPCL grade acetonitrile, 95% in deionised water. Sixteen different PAH compounds were detected. Recoveries were determined from an external standard PAH mixture Supelco (48743) at spiked levels of 200 µg /100 ml wet weight.

$$\mu g\ PAH/g\ meat = \frac{\mu g\ PAH\ in\ sample\ injected \times vol.\ of\ sample\ (1\ ml) \times 2}{Extract\ vol.\ (20\ \mu l) \times weight\ of\ sample}$$

3.3.7.13. Estimation of total carbonyls. (Henick *et al.*, 1958)

Preparation of Carbonyl Free Benzene: To 1 litre of benzene 5g of 2, 4 dinitro phenyl hydrazine (DNPH) & 1g of trichloroacetic acid were added. The contents were refluxed for an hour & distilled in all glass apparatus. The first 50 ml of the distillate was discarded.

Preparation of Carbonyl Free alcohol: To 1 litre of alcohol about 7g of Aluminium dust & 10g of potassium hydroxide pellets were added. The mixture was refluxed for 1 hour & distilled in an all glass apparatus discarding the first 50ml of distillate.

Five g of meat was made moisture free and extracted in carbonyl free benzene to 50ml in a volumetric flask. Five ml of this pipette out into a 50 ml volumetric flask and to it 3 ml of 4.3% tri-chloroacetic acid (4.3 % in benzene) and 5 ml of 2,4-dinitro phenyl Hydrazine (0.05% in benzene) was added and incubated at 60° C for half an hour to convert free carbonyl into hydrazine. After cooling 10 ml of potassium hydroxide pellets (4% in ethyl alcohol) was added and

the volume was made up to 50 ml with ethanol. After 10 minutes, absorbency was measured at 480 nm using a spectrophotometer. A blank was prepared in the same manner substituting sample extract, instead with 5ml benzene. A standard curve was drawn using Valeraldehyde (50-250 µg) in 5ml of benzene instead of sample extract The total carbonyl was calculated with the help of standard curve & expressed as mg of Valeraldehyde per 100g of sample.

$$\frac{\text{Conc. in µg x First vol. made up (50 ml) x Final Vol. Made up (50 ml) x100}}{\text{1000 x weight of sample in mg x 5}}$$

3.3.7.14. Estimation of total phenols (AOAC, 2000) Phenols (5530) / 5-43

Steam distillable phenols were estimated by the direct photometric method. Steam distillable phenolic compounds react with 4- aminoantipyrine at pH 7.9± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is kept in aqueous solution and the absorbance is measured at 500 nm.

10 g sample is mascerated thoroughly with 50 ml distilled water and transferred into a distillation flask and indirectly steam distilled. 100 ml of the distillate is collected in a 250 ml beaker. To this 2.5 ml 0.5 N ammonium hydroxide solution is added and then the pH is immediately adjusted to 7.9 ± 0.1 with phosphate buffer. To this 1 ml 4 - amino antipyrine solution is added and mixed well. Then 1 ml of potassium ferricyanide solution is added and mixed well. 100 ml distilled water and a series of 100 ml phenol standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 flask 100 mL distillate, or a portion containing not more than 0.5 mg is prepared.

$$\text{Mg phenol/kg} = \frac{C \times D \times 1000}{E \times B}$$

Where B= mg of original sample, C=mg standard phenol solution, D= absorbance of sample, E= absorbance of standard phenol solution.

3.3.7.15. Determination of Texture Profile

The TPA method of Bourne (1978) based on compression of samples with Universal Testing Machine (Lloyd instruments LRX plus,) was used to objectively evaluate textural differences between treatments. Uniform size tuna samples from pouches were used for the analysis. The load cell used was a cylindrical probe of 50mm diameter with 50 N capacity. The samples were compressed twice to 40% of their original height; at a crosshead speed of 12 mm/min. Force by time data from each test were used to calculate mean values for the TPA parameters. The values for hardness 1 and 2 (the resistance at maximum compression during the 1^{st} and 2^{nd} compression), cohesiveness (ratio of the positive force area during the 2^{nd} compression to that during the 1^{st} compression of Area 2/Area 1), springiness (ratio of the time duration of force input during the 2^{nd} compression to that during the 1^{st} compression of length 2/length 1), and chewiness (hardness1 x cohesiveness x springiness in kg mm) were determined as described by Bourne (1978). At least five duplicates were done and average readings were taken.

3.3.7.16. Sensory Test (IS: 6273[II]-1971)

Sensory evaluation was based on characterization and differentiation of the various sensory characters such as appearance, colour, texture, odour and flavour. Score was given based on a ten-point scale by trained taste panel members (Annexure 1), as per guideline given by IS: 6273[II]-1971. Scores 9-10, 6-8, 4-5 and 1-3 were taken for excellent, good, fair and poor respectively for each of the sensory characteristic. The 10 point was given for prime quality product, whereas 4 was the limit for unacceptability after which the product was rejected. The characteristics covered under the taste panel were appearance, colour, odour, flavor, texture and overall acceptability. Texture characteristics such as succulence, toughness, fibrosity , firmness and chewiness were also studied.

3.3.8. Commercial sterility test (IS: 2168-1971)

The thermally processed samples were incubated at 37°C for 15 days and 55°C for minimum of 5 days. The incubated pouches were aseptically opened and 1-2g of the samples were taken by a sterilized forceps and inoculated into the sterilized fluid thioglycolate broth in test tubes. Little sterilized liquid paraffin was put on to the top of the broth to create anaerobic condition and incubated at 37°C and at 55° C.

3.3.9. Statistical analysis (SPSS, 2000)

The SPSS 10.00 (SPSS, 2000) statistical packaging was used for analysis of the experimental results. Sufficient numbers of samples were carried out for each analysis. Results were expressed as mean ± standard deviation. Analysis of variance was used to calculate significant difference ($p<0.05$) between different pouches and samples stored at ambient temperature and accelerated temperature.

Annexure 1:

SENSORY EVALUATION OF THERMAL PROCESSED SMOKED TUNA

Assessor :..... Date: .....

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

CHARACTERISTICS	A	B
Appearance		
Colour		
Odour		
Flavour		
Taste		
Texture		
i. Firmness		
ii. Fibrousness		
iii. Succulence		
iv. Toughness		
v. Chewiness		
Overall acceptability		

(Please score the sample characteristics according to the following scale)

Quality grade description for appearance, colour, odour, flavour, taste and overall acceptability)

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

Description for texture

Firmness:

Quality Grade Description	Score
Firm	9-10
Moderately Firm	6-8
Slightly Firm	4-5
Soft	1-3

Fibrousness:

Quality Grade Description	Score
Extremely tender	9-10
Moderate tender	6-8
Slight tender	4-5
Fibrous	1-3

Succulence:

Quality description	Score
Very juicy	9-10
Moderately juicy	6-8
Slightly juicy	4-5
Not juicy	1-3

Firmness:

Quality description	Score
Very juicy	9-10
Moderately juicy	6-8
Slightly juicy	4-5
Not juicy	1-3

Chewiness:

Quality description	Score
Very juicy	9-10
Moderately juicy	6-8
Slightly juicy	4-5
Not juicy	1-3

Comments

Signature

Results and Discussions

4.0 RESULTS AND DISCUSSION

4.1. Physical properties of the packaging material

The physical properties of the four flexible pouches used in the study are given in Table 6.

4.1.1. Thickness

The thickness of different pouches ranged from 101-118.8 μm for the three layer retort pouches and was 131 μm for the two layered pouch. The total thickness for indigenous opaque pouches (INOP) was 105μm, indigenous see through (INST) pouches 101 μm and 118.8 μm for imported see through (IMST) pouches. The thickness of pouches directly influences the mechanical performance, product protection and integrity of the pouches (Hemavathi *et al.*, 2002).

4.1.2. Tensile strength and elongation at break

The tensile strength was 450 kg/cm^2 in machine and cross direction for indigenous opaque pouches and 816 kg/cm^2 and 488 kg/cm^2 for the imported see through pouches. For the indigenous retort pouches it was 717 kg/cm^2 and 592 kg/cm^2 and for 2 layer pouches it was 316 kg/cm^2 and 292 kg/cm^2 respectively. Elongation at break in machine direction was 20, 95, 78, and 53 and in cross direction it was 20, 76, 68 and 44 for INOP, IMST, INST and INTL pouches respectively. Elongation at break of the packaging materials determines the resistance to rupture and breakage when subjected to tensile force. These properties of packaging materials are of utmost importance to perform well with form fill seal operations (Vijayalakshmi *et al.*, 2003). The laminates possessed good tensile strength and elongation at break for both machine direction and cross / transverse direction.

Table 6: Physical properties of different flexible pouches

Parameters	Indigenous Opaque (INOP)	Imported See Through (IMST)	Indigenous See Through (INST)	Indigenous Two layered (INTL)
	PEST/Al foil/ CPP	PEST aluminium oxide/ Nylon/CPP	PEST silicon dioxide/ Nylon/ CPP	PEST/CPP
Total thickness (μm)	105 ± 0 .02	118 ± 0. 2	101 ± 0.01	131 ± 0.01
Tensile strength (M D) kg/cm^2	450 ± 0.01	816 ± 0.01	717 ± 0.01	316 ± 0.01
Tensile strength (CD) kg/cm^2	450 ± 0.02	488 ± 0.03	592 ± 0.01	292 ± 0.01
Elongation at break (MD) %	20 ± 0.01	95 ± 0.01	78 ± 0.01	53 ± 0.02
Elongation at break (CD) %	20 ± 0.02	76 ± 0.01	68 ± 0.01	44 ± 0.01
Heat seal strength (MD) N/25 mm width	310 ±3.25	504 ± 0.01	538 ± 0.03	303 ± 0.01
Heat seal strength (CD) N/25 mm width	237.9±2.11	224.3±2.21	412 ± 0.01	286 ± 0.02
Processors Heat seal strength N/25 mm width	196.7±2.31	179.4±2.24	189.4±2.24	191±2.34
Bursting strength (psig)	30 ± 0.01	26 ± 0.01	29 ± 0.01	29 ± 0.01
Bond Strength (g / 10 mm)	184.3±0.21	149±0.04	123±0.04	100.4±0.11
Overall migration residue (mg/dm^2)				
a. Water extractives	0.80 ± 0.01	4.8 ± 0.01	3.2 ± 0.01	6.8 ± 0.01
b. n-heptane (66 ° C/ 2 hr)	1.64 ± 0.02	2.45± 0.02	1.65 ± 0.02	1.98 ± 0.01
Residual air after processing (ml/100g)	1.83 ± 0.04	1.73± 0.02	1.89 ± 0.03	1.64 ± 0.32

WVTR g/m^2/24 h at 90 ± 2 % RH & 37°C	0.2 ± 0.01	0.2 ± 0.03	0.86 ± 0.02	1.99 ± 0.01
OTR (cc/m^2/24 h at 1 atmosphere pressure difference at 24°C Temp.	0.2± 0.01	0.6 ± 0.01	2.0 ± 0.01	55 ± 0.01

*Each value is represented as the average ± standard deviation of at least 10 determinants.

Where PEST- Polyester, Al-Aluminium, CPP-Cast Polypropylene, MD-Machine Direction, CD- Cross Direction

4.1.3. Heat seal strength

Heat seal strength is one of the most important parameters of retort pouches, which provides the pouches good package integrity and shelf-life. Heat seal strength of pouches sealed at three sides by the manufacturer was measured for machine direction and cross direction, where as for processor seal, it was measured in one direction only. It was found that the heat seal strength of manufacturer's seal was higher than the processor seal. Heat seal strength expressed as percentage for the INOP, IMST, INST and INTL pouches were 310, 504, 538 and 303 in machine direction and 237, 224, 412 and 286 in cross direction respectively. The processor seal for opaque, imported see through, indigenous see through and two layer pouches were 196.7, 179.4, 189.4 and 191 respectively. However, in all the cases the values were well above the prescribed limits (Lampi, 1997; 1980).

4.1.4. Bond strength

Bond strength is one of the most important requirements for retort pouch, which prevents delamination of laminates during thermal processing. Lower value indicates easy delamination of the layers during thermal processing, which results in physical destruction of pouches and reduction of barrier properties of retort pouches. It has been observed from the results that the bond strength for the INOP, IMST, INST and INTL pouches were 184, 149,123 and 100 respectively.

4.1.5. Bursting strength

The bursting strength was 30 psi for opaque pouches, 26 psi for imported see through, 29 psi for indigenous see through and two layer pouches. Bursting strength combines tensile strength and tear resistance and serves as a rough guide to compare the packaging materials (Vijayalakshmi *et al.*, 2003). It has been observed from the results that all the pouches passed the bursting test by holding at above 25 psig for one minute.

4.1.6. Barrier properties

The oxygen transmission rate (OTR) of the indigenous opaque pouches imported see through, indigenous see through and two layer pouches were 0.2, 0.6, 2 and 55 cc/m^2/24 h at 1 atmosphere pressure at 24°C and the water vapour transmission rate (WVTR) were 0.2, 0.2, 0.86 and 1.99 g/m^2/24 h at 90 ± 2 % RH & 37°C. The OTR is a very important parameter to know the headspace oxygen of the packaged retort pouch (Kumar, 1994). Low barrier properties for oxygen of retort pouch allow the penetration of oxygen into the pouch, which finally leads to rancidity in the food product inside the processed pouch. It was observed that in opaque pouches the OTR rate was as low as 0.2 which indicated good barrier properties of the pouch. The higher OTR of 55 in two layer pouches indicated that the two layer pouches had higher permeability rates.

The WVTR value of retort pouch for INOP pouches were 0.2, which is very low and may be due to the presence of aluminum foil layer in the pouches. The WVTR rates for INST, IMST and INTL pouches were 0.86, 0.2 and 1.99 respectively. The low transmission rate for IMST pouches are due to high barrier property of aluminum oxide layer and nylon present in the IMST pouches.

4.1.7. Overall migration test

Bureau of Indian Standard specify migration levels of 10 mg/dm^2 or 60 ppm for finished materials (IS: 10910, 1984). The levels of water extractives and n-heptane extractives were 0.80 and 1.64 for INOP, 4.8 and 2.45 for IMST, 3.2 and 1.65 for INST and 6.8 and 1.98 for INTL pouches. The values obtained for

the pouches were well below the limits. All polymeric plastic compounds belong to higher molecular weight and are inert and have limited solubility in aqueous and non aqueous system. But most of the plastic in finished form contains some non-polymeric components, which may leach out from plastic to foods whenever direct contact occurs between food and plastic thereby contaminating the food product with the consequent risk of toxic hazard to the consumer (Gopal,2005) Since the migration of food is inevitable, various countries have formulated standards, which specify maximum limit of migration.

4.1.8. Process resistance of pouches

All retort pouches were subjected to process resistance test and were found to be suitable and no delamination and wrinkles were observed after treating the pouches at 121.1°C for 30 minutes.

4.1.9. Residual air test after processing

The residual air test after the thermal processing of tuna in different medium showed that the value was well below the prescribed limit of 2% as described by (Shappee *et al.*, 1980). If the limit is exceeding, it will affect the shelf life of the product leading to rancidity. The excess air expands during thermal processing and affects seal integrity.

4.2. Raw and smoked tuna characteristics

4.2.1. Yield and appearance of tuna

Tuna smoked with teak wood and coconut husks for 30 and 60 minutes were acceptable to the trained sensory panelists. However steaks smoked for 60 minutes were found to have a deeper colour. Coconut husks were selected due to the low cost, local availability and acceptance of the smoked product. The yield of tuna steaks from whole tuna was approximately about 40 %. During the hot smoking process loss in moisture was noticed and thereby the weight of the fish decreased. 1000 g raw tuna steaks weighed 700 g after smoking process. The smoked tuna steaks had a characteristic brown colour and were

found to be firmer to touch than raw steaks due to the formation of the pellicle on the surface of the steaks. Characteristic flavour and odour of smoked fish was also present. Smoking enhanced the keeping quality of tuna steaks when compared to raw steaks due to the reduction in moisture and deposition of phenolic compounds from wood.

4.2.2. Proximate composition of tuna during different stages of processing

The proximate compositions of tuna steaks during various stages of preparation of tuna in pouches are given in the Table 7. Moisture content of tuna was about 73.08%, protein 23.27 %, fat 0.38 % and ash 4.03%. The moisture content decreased after hot smoking. Similar loss in moisture was reported for sardines (Beaumonte & Castrillion, 1989).

Table 7. Proximate composition of raw, brined and smoked tuna steaks

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Raw	73.08 ± 0.47	23.27 ± 0.16	0.38 ± 0.02	4.03 ± 0.08
Brined	72.16 ± 0.39	23.61 ± 0.45	0.25 ± 0.03	4.89 ± 0.05
Smoked	66.04 ± 1.02	29.90 ± 0.97	1.17 ± 0.28	5.03 ± 0.06

* Each value is represented by the average ± standard deviation of at least 3 determinants.

In the present study the protein content increased from 23.27% in the raw to 29.90 % in the smoked fish. Smoking removes moisture from the meat leading to an increase in the protein, fat and ash content. The white meat of tuna used for smoking and thermal processing had a low initial fat content. This was mainly due to the removal of the red meat, as canned tuna is considered to be of inferior quality if it contains red meat (Castrillion *et al.*, 1996). Salt added during the brining process has contributed to the increase in the ash content in tuna after smoking.

4.2.3. Quality evaluation of tuna during different stages of processing

The quality parameters of raw, brined and smoked tuna are given in Table 8. The pH of raw tuna was 6.21 which decreased to 6.16 and 6.02 after brining and smoking. This decrease could be due to the presence of different smoke components like acids which get deposited on the fish during the smoking process. The total volatile base nitrogen (TVBN) content increased from 10.82 to 13.53 mg N/100g. The free fatty acid value (FFA) also increased from 5.54 in the raw to 6.89 after smoking due to higher hydrolytic rancidity at elevated temperature. Similar results have been reported by Medina *et al.*, (1995) where precooking process increases the FFA content. The values of TBA which are compounds of secondary oxidation also showed an increasing trend from 0.23 mg malonaldehyde/kg in raw to 0.30 mg malonaldehyde/kg in smoked tuna. Lipid oxidation occurs at elevated temperatures in the presence of oxygen and hence the higher values for the smoked tuna

Table 8: Quality parameters of raw, brined and smoked Tuna

Parameter	Stages of Processing		
	Raw	Brined	smoked
pH	6.21 ±0.20	6.16± 0.07	6.02±0.01
Total volatile base nitrogen (mg N/100g)	10.82±0.74	11.63± .29	13.53±0.47
Free fatty acid (% oleic acid)	5.54 ± 0.32	6.34± 0.87	6.89± 0.33
Thiobarbituric acid (mg malonaldehyde /kg)	0.23±0.05	0.30± 0.01	0.30 ± 0.1

* Each value is represented by the average ± standard deviation of at least 3 determinants.

4.2.4. Changes in biogenic amines in tuna during different stages of processing

The changes in the biogenic amines namely Putrescine (PU), Cadaverine (CA), Spermidine (SD), Spermine (SM), Tyramine (TY), Agmatine (AG) and Histamine (HI) are given in Table 9.

Table 9: Changes in Biogenic amines of raw, brined and smoked tuna

	Stages in Processing		
Biogenic amines	**Raw (ppm)**	**Brined (ppm)**	**Smoked (ppm)**
Cadaverine	0.23±0.08	0.36±0.01	0.26±0.11
Putrescine	0.10±	0.05±0.1	0.17±0.02
Spermidine	0.16±0.08	0.07±0.03	0.16±0.02
Spermine	0.22±0.01	0.16±0.05	0.13±0.01
Tyramine	0.18±0.13	0.12±0.01	0.15±0.02
Agmatine	0.26±0.07	0.26±0.00	0.23 ±0.04
Histamine	4.67±1.05	5.19±0.65	5.89±0.81

* Each value is represented by the average ± standard deviation of at least 3 determinants.

The level of histamine in the raw tuna sample was 4.67 ppm. Histamine in the raw tuna is produced by the action of bacterial histidine decarboxylase on the amino acid histidine and environmental conditions. (Lenistea, 1973). Other biogenic amines of relevance here are putrescine from the amino acid ornithine, cadaverine from lysine and spermine and spermidine from arginine. Spermine and spermidine are usually lost during decomposition of tuna. The tuna used for the experiments were kept in ice (±2°C) from the time it was caught onboard till it

reached the processing facility and hence the initial level of histamine was very low. This is in agreement with the findings of Frank *et al.*, (1981) where low levels of histamine were obtained in iced tuna. Slight increase in the levels of histamine to 5.19 ppm after brining and to 5.89 ppm after smoking was also observed. Poulter, (1988) reported that hot smoking practically sterilizes the product, but does not destroy the histamine already formed. The levels of cadaverine and putrescence were very low in the raw (0.23 ppm and 0.10 ppm), brined (0.36 ppm and 0.05 ppm) and smoked tuna (0.26 ppm and 0.17 ppm). High levels of putresine and cadaverine occur in toxic fish (Arnold & Brown, 1978) and low levels in non toxic fish (Mietz & Karmas, 1977). The results obtained in the present study show that tuna used was of prime quality.

The level of spermidine in raw tuna was 0.16 ppm which remained the same after smoking the fish. However in the case of spermine the level decreased from 0.22 ppm in raw to 0.13 ppm in smoked. Tyramine levels in raw tuna were found to be 0.18 ppm which decreased to 0.15 ppm after smoking. Spermine and spermidine are biogenic amines which are inherent in tuna (Maijala *et al.*, (1995) and Hernandez-Jover, *et al.*,(1996). Agmatine levels were also low in the raw and smoked tuna. The levels increased to 0.23 ppm from 0.16 ppm after smoking. Similar trend has been reported by Veciana-nogules *et al.*, (1997).

4.2.5. Polyaromatic hydrocarbon (PAH) components of wood smoke

4.2.5.1. PAH content of tuna smoked with different woods.

The Polyaromatic hydrocarbon (PAH) compounds present in tuna smoked with different types of woods are given in Table 10. Saw dust from *Tectona grandis* (teak), *Callicarpa tomentosa* (*Cheruteak*), *Anacardium occidentale* (Cashew), *Buchania axillaries* (*Kolamavu*), *Acacia auriculiformis* (Acacia), *Terminalia paniculata* (*Maruthu*), *Artocarpus hirsutus* (Anjily) and husks from *Cocos nucifera* (coconut) were used for smoking tuna. The duration of smoking was for 60 minutes and varied levels of BaP content were seen in the tuna. The highest was recorded in Anjily 4.06 µg/kg followed by Acacia 3.63 µg/kg, and Coconut husks 1.48 µg/kg. The lowest level was recorded in Maruthu 0.08 µg/kg.

Table 10. PAH levels in tuna smoked with different types of wood

PAH Components µg/kg	Types of wood							
	Teak	Cheruteak	Cashew	Kolamavu	Acacia	Coconut	Maruthu	Anjily
Anthracene	N D	0.14 ± 0.02	0.15±0.07	0.03±0.07	0.62	N D	0.08	0.08
Fluoranthene	N D	0.28 ± 0.06	0.35±0.07	N D	0.014	1.8 ± 0	0.18	0.18
Pyrene	0.05±0.01	1.45 ± 0.04	1.40±0.21	0.03±0.001	0.661±0.05	9.20 ± 4.1	1.05	1.05
Benzo-a-anthracene	0.56	0.80 ± 0.11	1.26±0.02	0.01	0.63±0.01	3.53±1.12	0.58	0.60±0.03
Chrysene	N D	0.02	0.03±0.01	N D	N D	41	N D	N D
Benzo-b-Fluoranthene	1.14±0.13	53 ± 15.31	70.7±1.94	2.28±0.01	56.62±0.37	2 ± 0.75	34.65	33.32±1.87
Benzo-k-Fluoranthene	N D	N D	N D	N D	N D	0.83 ± 0.03	N D	N D
Benzo-a-Pyrene	0.45± 0.02	0.26 ± 0.06	0.56±0.02	0.25±0.02	3.63 ± 3.70	1.48 ± 0.13	0.08	4.06
Dibenzo-anthracene	N D	N D	0.44±0.09	N D	N D	1.9 ± 0.05	0.05	0.04
Benzo-ghi-perylene	N D	N D	N D	N D	N D	6 ±1.7	N D	N D
Indeno pyrene	N D	0.05 ± 0.0	N D	N D	N D	1.9 ± 1.48	0.025	0.03

Each value is represented by the average ± standard deviation of at least 3 determinants.

N.D. Not detected

The major PAH compounds studied in the smoked tuna in different medium are Anthracene (Ant), Fluoranthene (Flu), Pyrene (Py), Benzo-a-anthracene (BaA), Chrysene (Chr), Benzo-b-fluoranthene (BbF), Benzo-k-fluoranthene(BkF), Benzo-a-pyrene (BaP), Dibenzo-anthracene(DahA), Benzo-ghi-perylene (BghiP), and Indeno pyrene (Icd,P).

Benzo-a-pyrene (BaP) is accepted as the indicator of total PAH presence in smoked foods. A toxic equivalency factor (TEF) has been developed by Traag *et al.*, (2001) which showed BaP to have the highest carcinogenic potential. Benzo-a-Pyrene and Dibenzo-anthracene (DahA) are classified as "probably carcinogenic to humans" (group2A) by the International agency for research on Cancer (IARC) and has a total equilency factor (TEF) of 1. Benzo-a-anthracene (BaA), which is the third PAH in group 2A and the "possibly carcinogens" like Benzo-b- Fluoranthene (BbF), Benzo-k-fluoranthene (BkF), and Indeno pyrene (Icd,P) has a TEF of 0.1. Chrysene (Chr), Fluoranthene (Flu), Acenaphthelene has a TEF of 0.01 and Acenaphthelene, Phenanthrene and Pyrene has a TEF of 0.001. These 6 PAH compounds do not have any indication of toxicity (Jira, 2003).

4.2.5. 2. Polyaromatic hydrocarbons in raw, brined and smoked tuna

The levels of individual PAHs compounds in raw, brined and smoked tuna are presented in Table 11. BAP was not detected in the raw and brine tuna taken for this study. The tuna which was smoked at 75°C for a period of 60 min was found to contain 1.48 µg/kg of BaP. DahA which is also a potential carcinogen was found to be present to a level of 1.9 µg/kg in the smoked tuna. Various authors studied the levels of BaP in different types of hot smoked fish prepared using traditional smoke houses. The increase in BaP content in this study is in agreement with finding of similar studies on smoked fish. Zabik *et al.*, (1996) reported that lean and fat trout fillets smoked at 82°C contained BaP to the level of 5.12- 8.43 µg/kg. The BaP content smoked at different temperatures was found to be 2.6-3.7 µg/kg in skinned dogfish, 1.5-3.7 µg/kg in halibut muscle and 1.1-2.4 µg/kg in sprat muscle. (Steinig & Meyer, 1976), 0.3-3.9 µg/kg in eel

muscle, 3.6 µg/kg in halibut muscle and 0.5-2.4 in mackerel muscle respectively (Karl & Leinemann, 1996). Fluorene and anthracene were not detected in any of the samples. Levels of DahA, BaA, BbF, BkF, and Icd, P in the smoked fish were 1.9, 3.53, 0.83, 2 and 9.2 µg/kg respectively.

Table 11. PAH levels in raw, brined and smoked tuna

Components	Raw(µg/kg)	Brine(µg/kg)	Smoked(µg/kg)
Anthracene	N D	N D	N D
Fluoranthene	0.02 ± 0.02	0.01 ± 0.01	1.8
Pyrene	0.23 ± 0.12	0.21 ± 0.16	9.20
Benzo-a-anthracene	0.01 ± 0.01	0.01 ± 0.01	3.53±1.59
Chrysene	0.26	0.20 ± 0.12	39.95±1
Benzo-b- Fluoranthene	0.01	0.01	2 ± 0.75
Benzo-k- Fluoranthene	N D	N D	0.83 ± 0.03
Benzo-a-Pyrene	N D	N D	1.48 ± 0.18
Dibenzo-anthracene	0.01 ± 0.01	N D	1.9 ± .05
Benzo-ghi- perylene	0.02 ± 0.02	0.01	6 ± 0.99
Indenopyrene	0.03 ± 0.01	0.03 ± 0.01	1.9±1.48

N.D. Not detected

Data are average of duplicate analysis

The development of PAH depends on the smoking method employed. It is seen that the smoke generation temperatures influence the PAH generation (Toth & Blaas, 1972). At higher temperatures the PAH contents are produced in greater quantities. Restricting the oxygen supply and moistening the wood source during smoking reduce the smoke generation temperature and thereby the PAH levels (Raja *et al.,* 1980 and Potthast, 1979). PAH in smoked fish are influenced by wood

source (Larsson, 1982), composition of wood (Maja, 1986) and wood based charcoal (Kushwaha, 1985).

4.2.6. Total Carbonyls

4.2.6.1. Total carbonyl content of tuna smoked with different woods.

The total carbonyl content in tuna smoked with different types of wood used in the study is given in Table 12.

Table. 12. Changes in total carbonyl content for smoked tuna using different types of wood.

Type of wood	Carbonyl content (mg/kg)
Cheruteak	1.42 ±0.1
Cashew	0.31 ±.09
Kolamavu	0.59 ±0.06
Acacia	2.10 ±0.07
Coconut	1.01 ±0.01
Maruthu	0.27 ± 0.07
Anjily	0.43 ±0.06
Teak	1.61 ±.01

* Each value is represented by the average ± standard deviation of at least 3 determinants.

Tuna smoked with acacia and teak wood recorded the high values of 2.01 and 1.61 mg/kg followed by *cheruteak* and coconut wood which gave values of 1.41 and 1.01 mg/kg respectively. *Maruthu, kolamavu, anjily* and cashew had low values of total carbonyls ranging from 0.42 mg/kg to 0.27 mg/kg. The carbonyls

account for the largest number of compounds identified in wood smoke (Maga, 1988) and are produced due to the thermal decomposition of cellulose and hemi cellulose (Kim *et al.*, 1974). The carbonyls in smoke are said to be major contributors to the color of the product. The carbonyls in the smoke react with the amino groups in the fish protein on the surface of the fish to give the characteristic colour. Each wood have different carbonyl compounds and hence vary in their color. In this study coconut husks were found to have a pleasing odor and acceptable taste. Solanki *et al.*, (1990) in their studies on eel smoked with different types of wood described the color of eel smoked with coconut husk to be pale yellow whereas that smoked with teak and acacia to be rich red. They also suggested a combination of acacia and teak wood to have a rich yellow colour but found that the odor in teak wood and acacia were not acceptable since it was acrid.

4.2.6.2. Total carbonyls in raw, brined and smoked tuna

The changes in total carbonyl compounds in raw, brined and smoked tuna are given in Table 13.

Table 13. Changes in total carbonyl values during different stages of processing

	Processing Stages		
	Raw	**Brined**	**Smoked**
Carbonyl content(mg/kg)	0.14±0.01	0.18± 0.02	1.01±0.02

* Each value is represented by the average ± standard deviation of at least 3 determinants.

The carbonyl values increased from 0.14 mg/kg to 1.01 mg/kg after the smoking process. Since pyrolysis of wood at high temperatures produces smoke containing a number of aldehydes and ketones, they get deposited on the fish and hence the increase in these compounds is seen on the fish. The brown colour

reactions are time and temperature bound, where at high temperature the reactions are faster and slower at lower temperatures (Rozum, 1998)

4.2.7. Total Phenols

4.2.7.1. Total Phenol content of tuna smoked with different types of wood

The steam distillable total phenol content of tuna smoked with different types of wood is given in Table 14.

Table 14: Total phenol content of tuna ssmoked with different woods.

Type of wood	Phenol content (mg/kg)
Cheruteak	0.49± 0.02
Cashew	0.31± 0.02
Kolamavu	0.52± 0.02
Acacia	0.32± 0.02
Coconut	0.63±0.03
Maruthu	0.42± 0.02
Anjily	0.39± 0.02
Teak	0.56± 0.02

Each value is represented by the average ± standard deviation of at least 3 determinants.

Coconut husk had the highest phenol content of 0.62 mg/kg and cashew had the lowest with 0.31 mg/kg. In tuna smoked with *cheruteak* it was (0.49 mg/kg), *kolamavu* (0.52 mg/kg), acacia (0.32 mg/kg), *maruthu* (0.42 mg/kg), anjily (0.39 mg/kg) and teak 0.56 mg/kg. Phenols are the main contributors to wood smoke aroma (Knowles *et al.*, 1975 and Kashara and Nishibori, 1979). Phenols

responsible for the smoked flavour are from lignin which produces phenolic compounds like syringol and 2-6 dimethoxyphenol, eugenol, isoeugenol, guaicol, phenol and cresols (Gilbert & Knowles, 1975).

4.2.7.2. Total phenols in raw, brined and smoked tuna.

The changes in phenol during the various stages of processing are given in Table 15. Phenols were not detected in the raw and brine sample. After smoking the phenol content was 0.64 mg/kg. During smoking the total phenol content increases due to the deposition of phenolic compounds from the wood which is the source of smoke.

Table 15: Changes in total phenol content during different stages of processing.

	Processing Stages		
	Raw	**Brined**	**Smoked**
Phenol content(mg/ kg)	N.D	N.D	0.64± 0.02

* Each value is represented by the average ± standard deviation of at least 3 determinants.
N.D. Not detected

4.3. Thermal processing and heat penetration characteristics

The smoked tuna was thermal processed to Fo 10 in three different form i.e., tuna natural or dry pack without any liquid medium, tuna in oil and as tuna in brine. The heat processing was done using different pouches for all the three forms which showed that there was variation in the process time with regard to the type of pouches and filling media. Heat penetration characteristics like Fo and cook value of tuna in different medium processed to Fo value of 10 in opaque, imported see through, indigenous see through and two layer pouches were determined. Heat penetration characteristics at the slowest heating point should be specific, if parameters such as filled weight, head space, type of container, dimension of container, come up time of the retort, heating media and initial

temperature were uniform for a given product (Vanloey, 1994). The Fo recommended for fish and fish products ranges from 5-20 (Frott and Lewis, 1994). A Fo value of 10 gave a good product with desired sensory and textural characteristics (Ali *et al.*, 2005).

4.3.1. Heat penetration characteristics of smoked tuna in indigenous opaque pouches

The heat penetration characteristics of smoked tuna in different media in opaque pouches are given in Table.16.

Table 16. Heat penetration characteristics of smoked tuna packed in opaque pouches in different medium

INOP pouches	f_h(min)	J_h	J_c	f_h/U	g (°C)	B (min)	Total process time T_B (min)	Cook value (Cg) value (min)
Brine	14.50 (0.07)	0.90 (0.02)	1.07 (0.03)	1.41 (0.01)	0.57 (0.02)	29.46 (0.46)	32.00 (1.75)	75.61 (0.99)
Oil	16.50 (0.85)	1.08 (0.18)	1.3 (0.09)	1.72 (0.09)	0.95 (0.04)	34.54 (2.26)	38.02 (1.06)	86.83 (0.63)
Dry pack	17.00 (0.85)	1.37 (0.85)	1.05 (0.85)	1.51 (0.85)	0.72(0.85)	37.72 (0.85)	42.01 (0.85)	89.31 (0.85)

Each value is represented by the average ± standard deviation of at least 3 determinants. The value in brackets gives the standard deviation

Where fh = slope of heating curve Jh= lag factor of heating, Jc = lag factor of cooling, U= time in minutes for sterilization at retort temperature, g = final temperature deficit, B= Ball's process time, T_B = total process time and Cg = cook value

The heating rate index f_h increased from brine (14.5 min), oil (16.50 min) to dry pack (17 min). The ball process time was 29.46 min, 34.54 min and 37.72 min for tuna in brine, oil and dry pack. The total process time (T_B) for smoked tuna by adding 58% of come up time for tuna in brine was 32 min, tuna in oil was 38.02 min and tuna natural pack was 42.01 min. In smoked tuna in brine medium the cook value was 75.61min, in oil medium 86.83 min and dry pack 89.31min. Heat penetration was faster in tuna packed in brine medium followed by tuna in oil and the slowest was in dry pack. The other parameters like lag factor of heating (Jh) lag factor of cooling (Jc), time in minutes for sterilization at retort temperature (U), final temperature deficit (g) are also given in the table. The graphs depicting the Fo value, cook value, retort temperature and core temperature for tuna in brine, oil and dry pack are given in Figures 1-3 respectively.

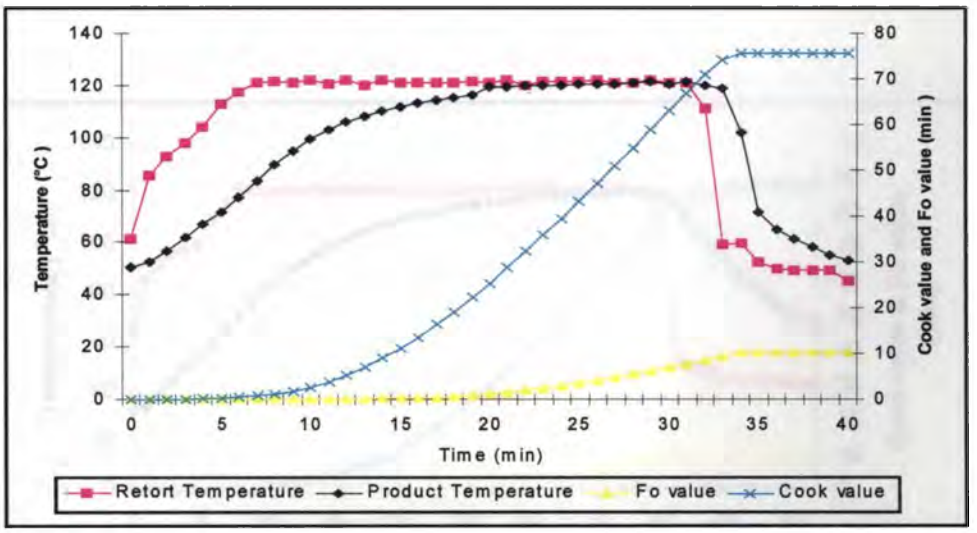


Fig. 1. Heat penetration characteristics of smoked tuna in brine in indigenous opaque pouches

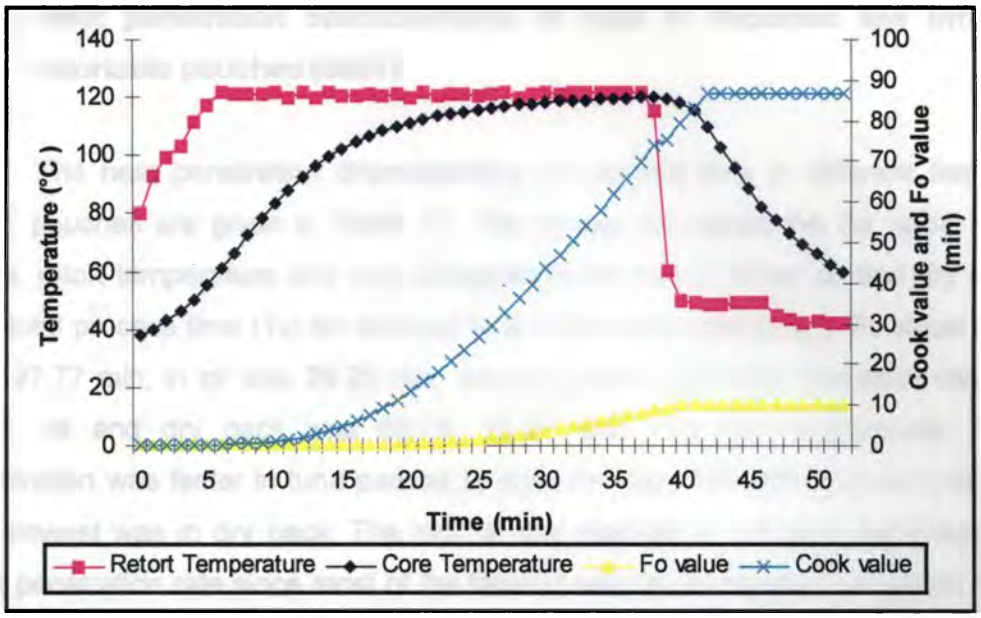


Fig. 2. Heat Penetration characteristics of smoked tuna in oil in indigenous opaque pouches

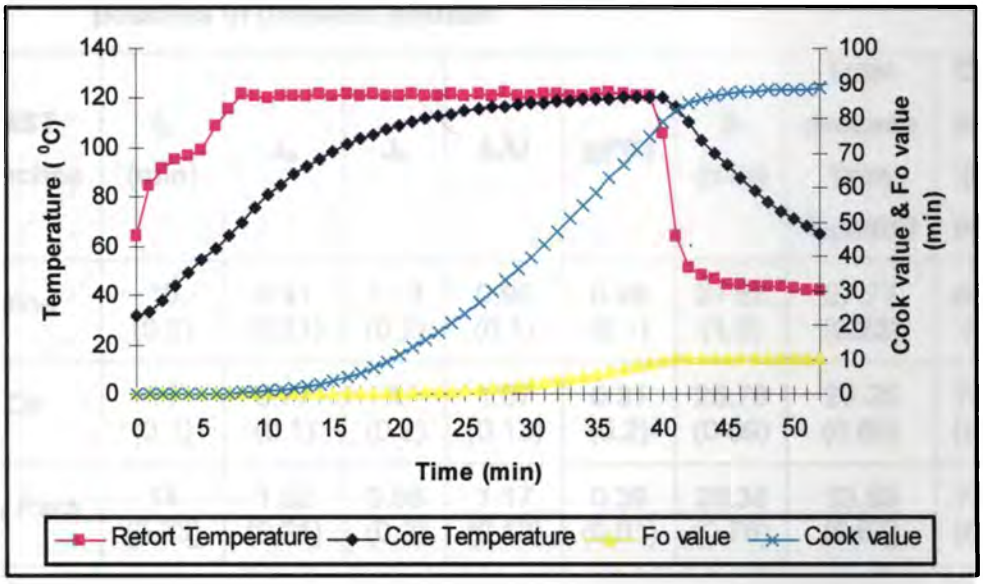


Fig. 3. Heat Penetration characteristics of smoked tuna dry pack in opaque pouches

4.3.2. Heat penetration characteristics of tuna in imported see through retortable pouches (IMST)

The heat penetration characteristics of smoked tuna in different forms in IMST pouches are given in Table 17. The figures 4-6 depict the Fo value, cook value, retort temperature and core temperature for tuna in brine, oil and dry pack. The total process time (T_B) for smoked tuna in brine processed to a Fo value of 10 was 27.77 min, in oil was 29.25 min, and dry pack 33.83 min. The cook value in brine, oil and dry pack was 69.74, 75.37 and 77.8 min respectively. Heat penetration was faster in tuna packed in brine medium followed by tuna in oil and the slowest was in dry pack. The lack of any medium in dry pack increased the heat penetration rate since most of the heating was by conduction unlike that of oil and brine where the heating is partly by convection too. The liquid medium transmitted heat faster than a solid pack.

Table 17. Heat penetration characteristics of tuna in imported see through pouches in different medium

IMST pouches	f_h (min)	J_h	J_c	f_h/U	g(°C)	B (min)	Total process time T_B (min)	Cook value (Cg) (min)
Brine	10 (0.2)	0.91 (0.21)	1.13 (0.2)	0.96 (0.1)	0.28 (0.1)	24.87 (1.0)	27.77 (0.23)	69.74 (0.1)
Oil	11 (0.3)	0.77 (0.1)	1 (0.2)	1.07 (0.13)	0.31 (0.2)	25.78 (0.09)	29.25 (0.65)	75.37 (0.67)
Dry Pack	14 (0.23)	1.02 (0.21)	0.96 (0.2)	1.17 (0.12)	0.39 (0.01)	28.35 (0.76)	33.83 (0.67)	77.80 (0.56)

* Each value is represented by the average ± standard deviation of at least 3 determinants. The value in brackets gives the standard deviation

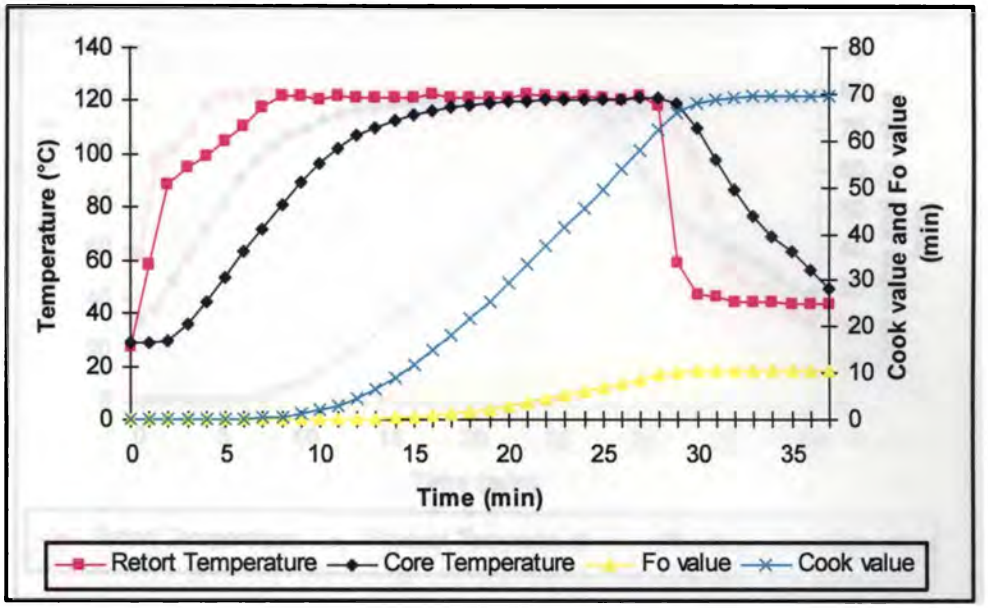


Fig. 4. Heat Penetration characteristics of smoked tuna in brine in imported see through pouches

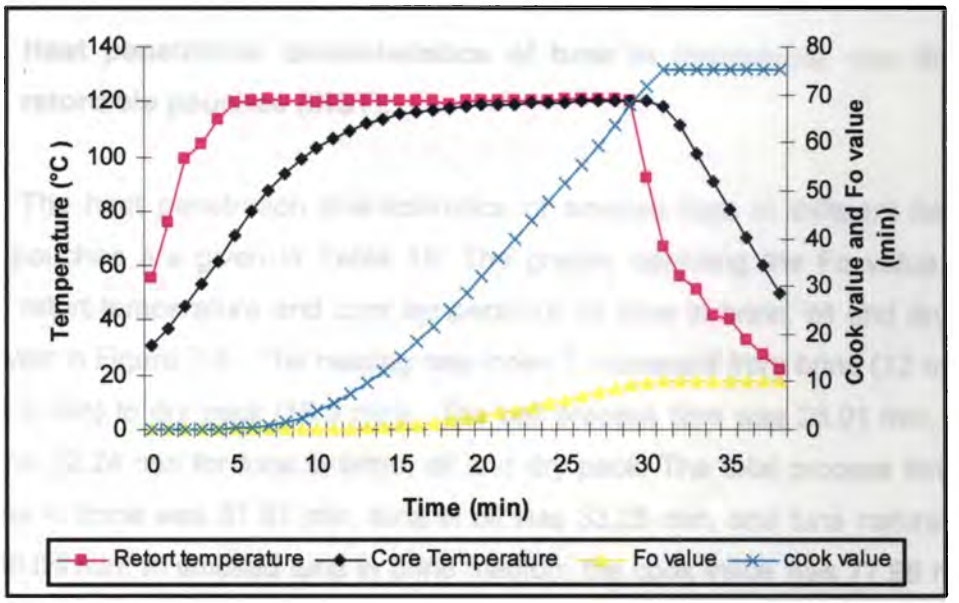


Fig. 5. Heat Penetration characteristics of smoked tuna in oil in imported see through pouches

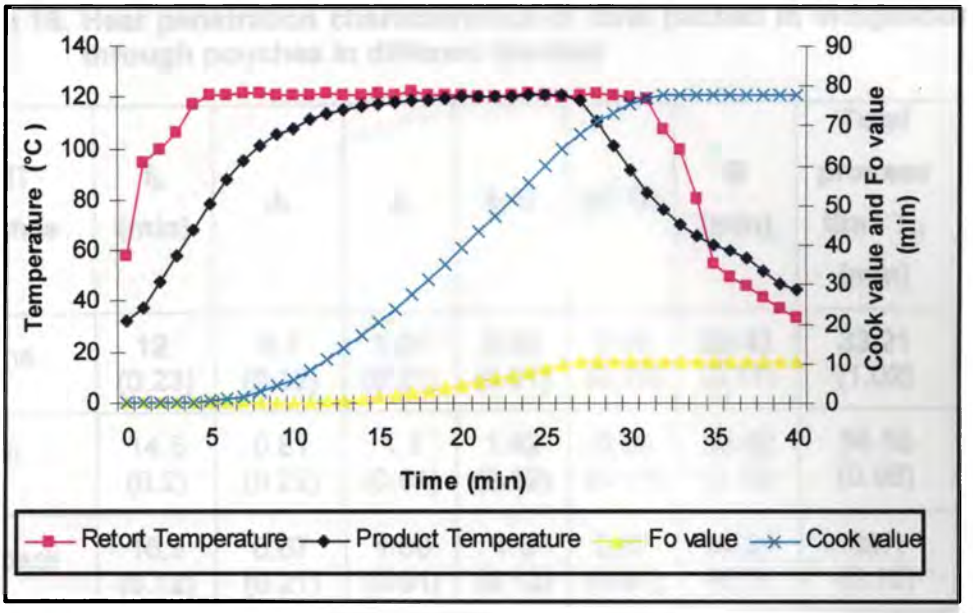


Fig. 6. Heat Penetration characteristics of smoked tuna dry pack in imported see through pouches

4.3.3. Heat penetration characteristics of tuna in indigenous see through retortable pouches (INST).

The heat penetration characteristics of smoked tuna in different forms in INST pouches are given in Table 18. The graphs depicting the Fo value, cook value, retort temperature and core temperature for tuna in brine, oil and dry pack are given in Figure 7-9. The heating rate index f_h increased from brine (12 min), to oil (14.5 min) to dry pack (16.5 min). The ball process time was 28.91 min, 30.62 min and 32.24 min for tuna in brine, oil and dry pack. The total process time (T_B) for tuna in brine was 31.91 min, tuna in oil was 33.25 min, and tuna natural pack was 38.04 min. In smoked tuna in brine medium the cook value was 77.98 min, in oil medium 80.26 min and dry pack 84.05 min. Heat penetration was faster in tuna packed in brine medium followed by tuna in oil and the slowest was in dry pack.

Table 18. Heat penetration characteristics of tuna packed in indigenous see through pouches in different Medium

INST pouches	f_h (min)	J_h	J_c	f_h/U	g(° C)	B (min)	Total process time T_B (min)	Cook value (Cg) (min)
Brine	12. (0.23)	0.7 (0.12)	1.01 (0.21)	0.98 (0.01)	0.43 (0.12)	28.43 (0.11)	33.21 (1.02)	77.98 (0.56)
Oil	14.5 (0.2)	0.81 (0.22)	1.1 (0.01)	1.42 (0.12)	0.60 (0.11)	32.62 (0.12)	36.35 (0.98)	80.26 (0.32)
Dry pack	16.5 (0.12)	0.67 (0.21)	1.00 (0.01)	1.6 (0.12)	0.67 (0.01)	34.24 (0.1)	39.1 (0.12)	84.05 (0.65)

* Each value is represented by the average ± standard deviation of at least 3 determinants . The value in brackets gives the standard deviation

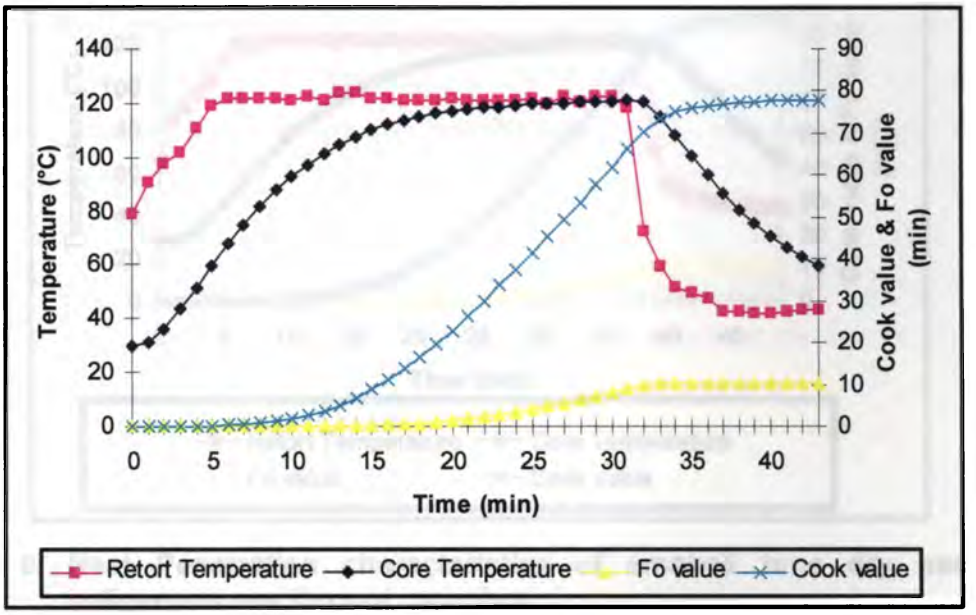


Fig. 7. Heat Penetration characteristics of smoked tuna in brine in indigenous see through pouches

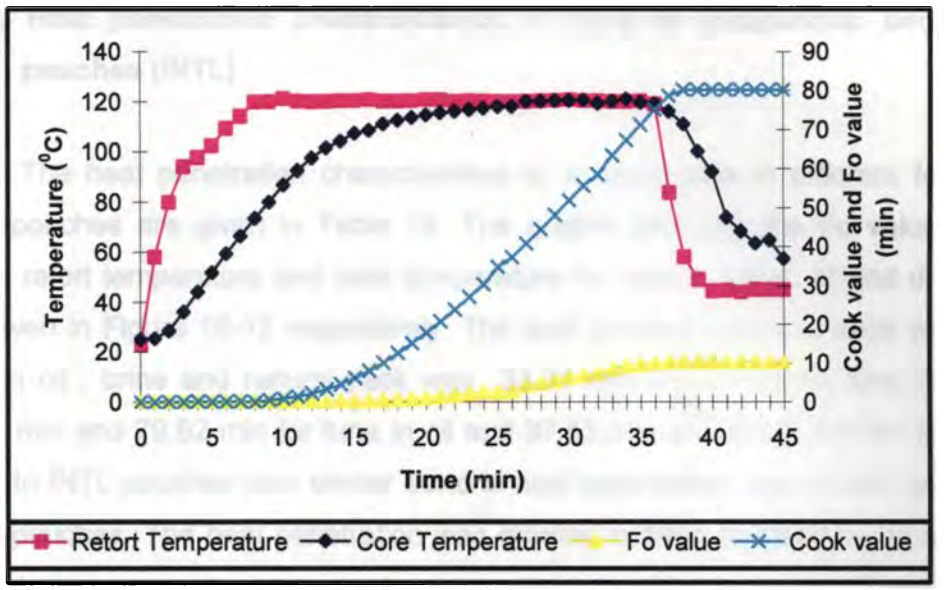


Fig. 8. Heat Penetration characteristics of smoked tuna in oil in indigenous see through pouches

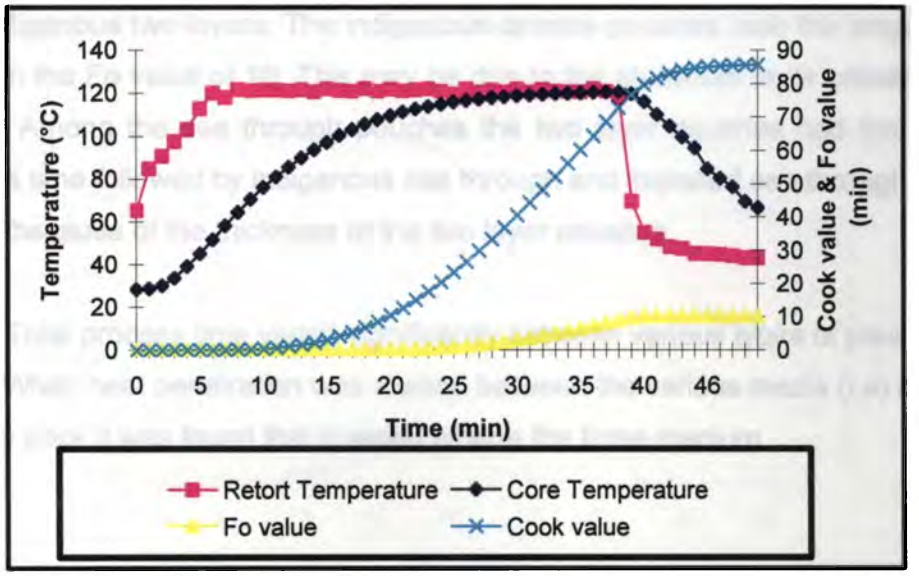


Fig. 9. Heat Penetration characteristics of smoked tuna dry pack in indigenous see through pouches

4.3.4. Heat penetration characteristics of tuna in indigenous two layer pouches (INTL)

The heat penetration characteristics of smoked tuna in different forms in INTL pouches are given in Table 19. The graphs depicting the Fo value, cook value, retort temperature and core temperature for tuna in brine, oil and dry pack are given in Figure 10-12 respectively. The total process time and cook value for tuna in oil , brine and natural pack was 33.31 min and 74.14 for tuna in brine, 36.35 min and 79.82 min for tuna in oil and 37.53 min and 82.48 min for tuna dry pack. In INTL pouches also similar trend in heat penetration rate is seen as in the other pouches. The heat penetration was quicker in brine followed by oil and dry pack.

When we compare the four different pouches we see that total process time was lowest for imported see through pouches followed by indigenous see through and indigenous two layers. The indigenous opaque pouches took the longest time to attain the Fo value of 10. This may be due to the aluminum layer present in the pouch. Among the see through pouches the two layer pouches had the highest process time followed by indigenous see through and imported see through. This is mainly because of the thickness of the two layer pouches.

Total process time varied significantly between various types of pouches ($p \leq 0.01$). When heat penetration was studied between the various media (*i.e*) oil, brine and dry pack it was found that it varied among the three medium.

Table 19: Heat penetration studies of tuna packed in indigenous two layer pouches in different medium

INTL pouches	f_h (min)	J_h	J_c	f_h/U	g (°C)	B (min)	Total process time T_B (min)	Cook value (Cg) (min)
Brine	13 (0.12)	0.94 (0.11)	1.02 (0.21)	0.98 (0.01)	0.44 (0.42)	29.73 (0.21)	33.21 (1.21)	74.14 (1.76)
Oil	15 (0.20)	0.88 (0.23)	1.01 (0.01)	0.99 (0.02)	0.52 (0.32)	32.87 (0.11)	36.35 (1.01)	79.82 (0.68)
Dry pack	16 (0.34)	0.77 (0.11)	0.10 (0.02)	1.01 (0.01)	0.63 (0.11)	32.80 (1.9)	37.53 (0.98)	82.45 (0.19)

* Each value is represented by the average ± standard deviation of at least 3 determinants. The value in brackets gives the standard deviation

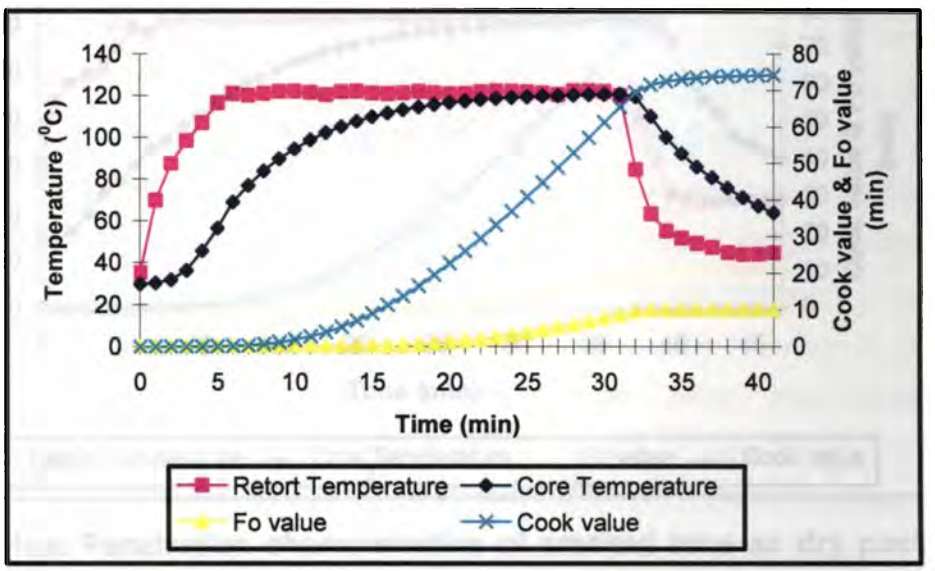


Fig. 10. Heat Penetration characteristics of smoked tuna in brine in indigenous two layer pouches

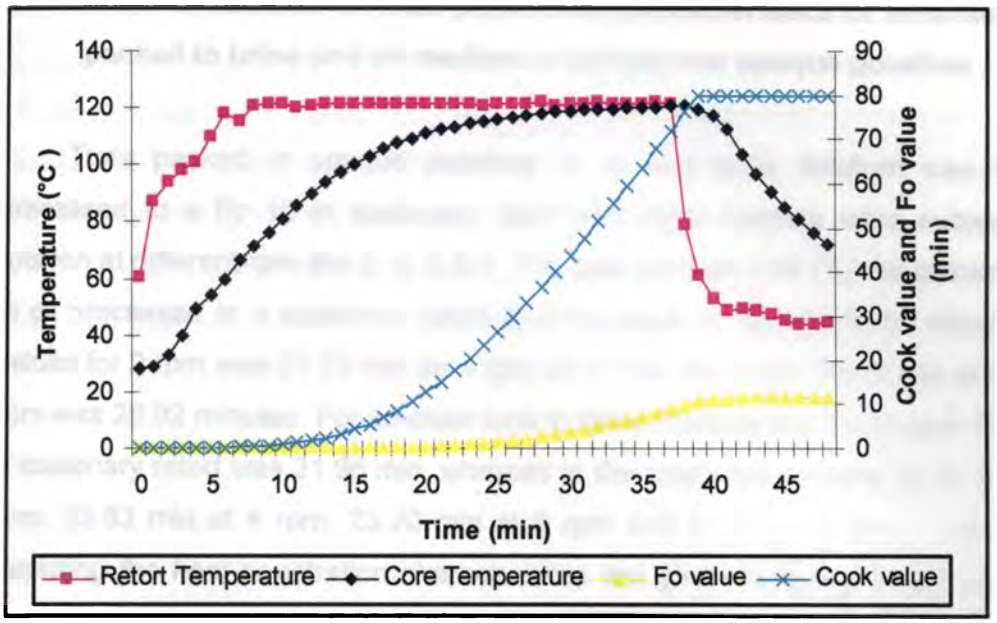


Fig. 11. Heat Penetration characteristics of smoked tuna in oil in indigenous two layer pouches

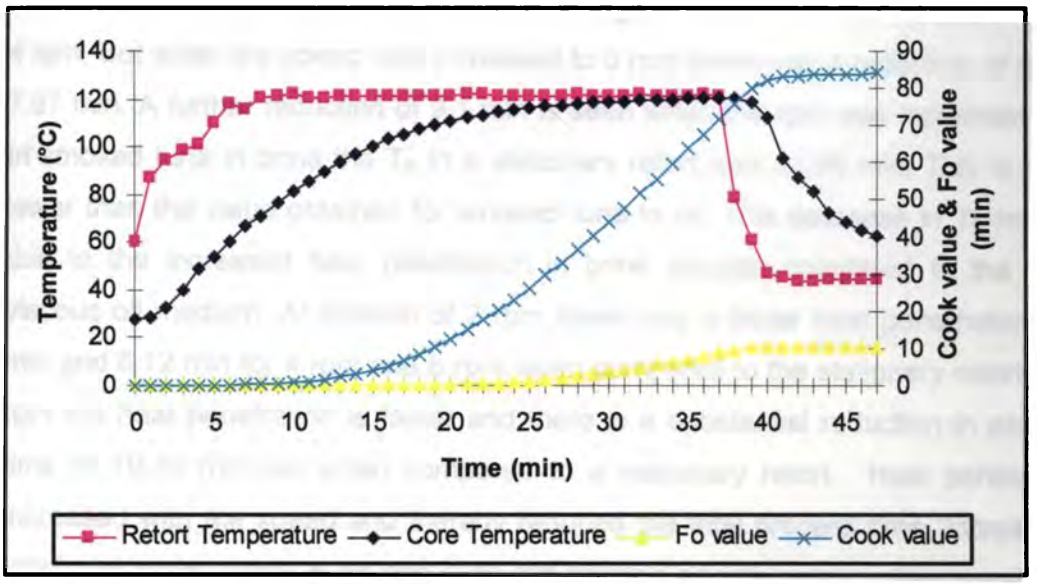


Fig. 12. Heat Penetration characteristics of smoked tuna as dry pack in indigenous two layered pouches

4.3.5. Effect of rotation on heat penetration characteristics of smoked tuna packed in brine and oil medium in indigenous opaque pouches

Tuna packed in opaque pouches in oil and brine medium was first processed to a Fo 10 in stationary retort and other batches were subjected to rotation at different rpm like 2, 4, 6 & 8. The total process time (T_B) for smoked tuna in oil processed in a stationary retort to a Fo value of 10 was 38.02 minutes. T_B values for 2 rpm was 31.73 min for 4 rpm 31.11 min for 6 rpm 30.05 min and for 8 rpm was 28.92 minutes. For smoked tuna in brine medium the T_B to reach Fo 10 in a stationary retort was 31.95 min, whereas in the rotary retort it was 25.95 min at 2 rpm, 23.83 min at 4 rpm, 23.20 min at 6 rpm and 21.60 at 8 rpm. The graphs depicting the heat penetration characteristics are given in figures 13-20 and heat penetration characteristics in Tables 20 and 21. In both the cases there was a reduction in process time up to 8 rpm. The T_B decreased with increase in rotation. In smoked tuna in oil medium, when compared to the stationary retort there was a reduction of 6.29 min for 2 rpm rotation. For 4 rpm there was a reduction of 6.91 min. It is observed that there is no much change in T_B with rotation speed of 2 and 4 rpm. But when the speed was increased to 6 rpm there was a reduction of nearly 7.97 min. A further reduction of 9.1 min is seen when the rpm was increased to 8. In smoked tuna in brine the T_B in a stationary retort was 31.95 min. This is much lower than the value obtained for smoked tuna in oil. This decrease in T_B may be due to the increased heat penetration in brine solution compared to the more viscous oil medium. At rotation of 2 rpm there was a faster heat penetration of 6 min and 8.12 min for 4 rpm and 6 rpm when compared to the stationary retort. At 8 rpm the heat penetration is faster and there is a substantial reduction in process time of 10.35 minutes when compared to a stationary retort. Heat penetration increased with the speed and thereby reduced the total process time. Increase in rotational speed (rpm) in an end over end rotation or axial rotation has resulted in an increase in heat penetration rate in liquid and semi liquid foods, (Ali *et al.*, 2006; Berry *et al.*, 1979; Berry & Bradshaw, 1980; Berry & Bradshaw, 1982; Naveh & Kopelman, 1980; Berry & Dickerson, 1981 and Berry & Kohnhorst, 1985). Vanloey

et al., (1994) has found that increasing the rate of rotation is limited, since at higher rotational speed of 20 rpm there is breakage of the product. Heat penetration factor (fh) decreased in the rotary retort with relation to faster heat penetration. This is due to fact that liquid media can move more effectively through the agitated cans, thereby increasing the heat penetration.

Table-20 Heat penetration characteristics of smoked tuna in oil processed to a Fo of 10 in opaque retort pouches

Rotation speed (rpm)	f_h (min)	J_h	J_c	f_h/U	g	B (min)	Total process time T_B (min)	Cook value (Cg) (min)
No rotation	17.5 (0.85)	1.08 (0.18)	1.3 (0.09)	1.72 (0.09)	0.95 (0.04)	34.54 (2.26)	38.02 (1.06)	89.83 (0.63)
2 rpm	12 (0.48)	1.01 (0.18)	1.03 (0.17)	1.17 (0.17)	0.41 (0.05)	28.25 (3.76)	31.73 (0.92)	72.26 (0.20)
4 rpm	11 (0.48)	1.2 (0.06)	1.06 (0.02)	1.07 (0.07)	0.33 (0.06)	27.63 (1.83)	31.11 (1.99)	70.77 (0.98)
6 rpm	10.5 (0.07)	1.13 (0.20)	1.04 (0.12)	1.04 (0.20)	0.3 (0.05)	26.57 (2.22)	26.57 (1.00)	67.72 (0.65)
8 rpm	10 (0.82)	1.27 (0.07)	1.08 (0.02)	0.98 (0.19)	0.28 (0.06)	26.02 (2.71)	26.02 (1.01)	65.08 (1.00)

* Each value is represented by the average ± standard deviation of at least 3 determinants.

The value in brackets gives the standard deviation

Kumar and Bhattacharya, (1991) reported that heat penetration is faster in rotating retorts and warm liquid foods. The parameters like fh/U and g depend on the heat penetration factor. fh /U and g show a decreasing trend with increase in rotational speed in both tuna in oil and brine. Cook value which is a measure of tenderness of the product was also measured. The values showed a decrease

when there was an increase in speed of rotation. This is due to the lesser process time for increasing rotation.

Table 21: Heat penetration characteristics of smoked tuna in brine processed to a Fo of 10 in opaque retort pouches

Rotation speed (rpm)	fh (min)	Jh	Jc	fh/U	g	B (min)	Total process time T_B (min)	Cook value (Cg) (min)
No rotation	14.50 (0.07)	0.90 (0.02)	1.07 (0.03)	1.41 (0.01)	0.57 (0.02)	29.46 (0.46)	32.00 (0.75)	75.61 (0.99)
2 rpm	8.50 (0.08)	0.88 (0.01)	0.98 (0.02)	0.83 (0.02)	0.18 (0.02)	22.47 (0.58)	25.95 (1.48)	60.10 (1.50)
4 rpm	7.50 (0.02)	0.72 (0.01)	1.02 (0.02)	0.73 (0.02)	0.12 (0.02)	20.35 (0.31)	23.83 (1.02)	58.54 (0.43)
6 rpm	7.00 (0.13)	0.71 (0.01)	1.96 (0.03)	0.72 (0.01)	0.10 (0.03)	19.72 (0.33)	23.20 (0.99)	57.86 (0.29)
8 rpm	7.00 (0.08)	0.43 (0.01)	.95 (0.01)	0.54 (0.04)	0.10 (0.03)	18.12 (0.31)	21.60 (1.03)	56.00 (1.70)

* Each value is represented by the average ± standard deviation of at least 3 determinants

**The value in brackets gives the standard deviation

Comparing the heat penetration between the tuna packed in brine and oil medium, it was observed that the total process time is more for smoked tuna in oil than in brine due to the slower heat penetration in oil medium when compared to brine. Total process time was highest for tuna processed in the stationery retort and decreased with increase in rotation. This was observed in both brine and oil medium.

Statistical analysis showed that rotation had a significant effect on the cook value and total process time in the case of smoked tuna in oil and brine medium. The data pertaining to smoked tuna in oil subjected to stationary, 2 rpm, 4 rpm , 6 rpm and 8 rpm was analyzed and it was found that total process time and cook value varied significantly between different levels of rotation (R^2 = 0.99 for cook value and R^2 = 0.94 for total processing time). Tukey's test was performed to find the significance of mean difference between levels of rotations for cook value in smoked tuna in oil, which showed that rotation of 2 rpm and 4 rpm is significantly different ($P \leq .05$).from 6 rpm and 8 rpm for tuna in oil. For total process time, rpm 2 is significantly different from rpm 8.

The data pertaining to smoked tuna in brine subjected to stationary 2, 4, 6 and 8 was analyzed and it was found that total process time and cook value varied significantly between different levels of rotation. (R^2 = .99 for cook value and R^2 = 0.94 for total process time). Increasing the number of rotations to 2, 4, 6 and 8 rpm was having a significant impact on the total process time and cook value. ($P \leq .01$).

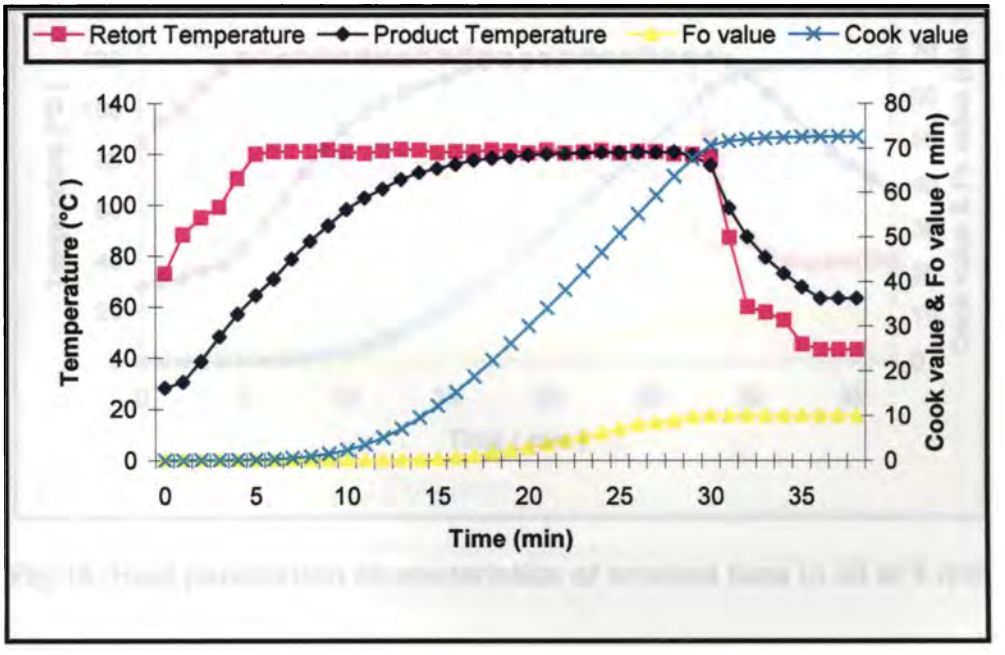


Fig 13. Heat penetration characteristics of smoked tuna in oil at 2 rpm

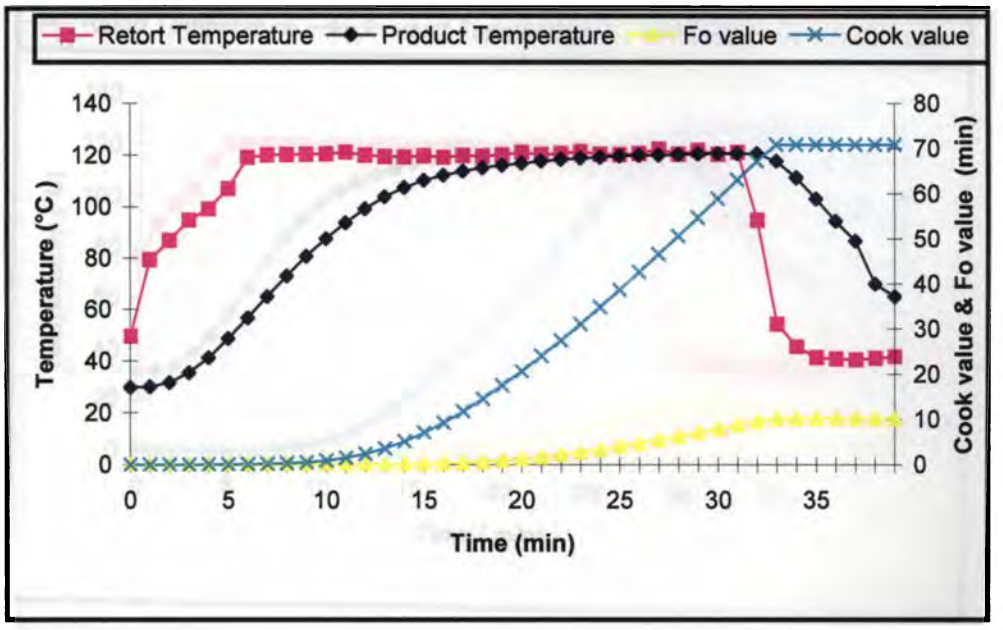


Fig. 14. Heat penetration characteristics of smoked tuna in oil at 4 rpm

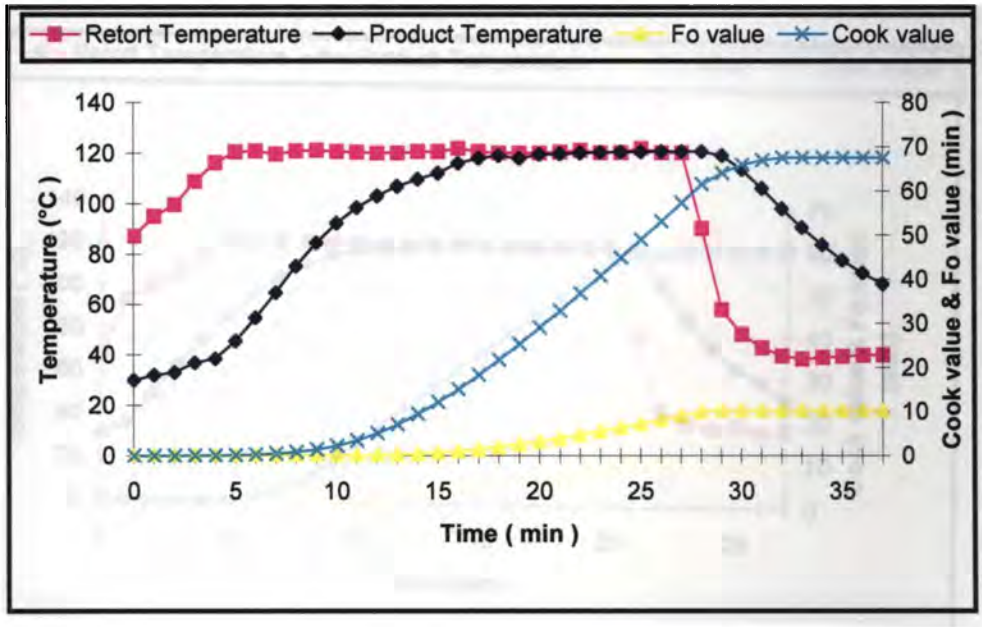


Fig 15. Heat penetration characteristics of smoked tuna in oil at 6 rpm

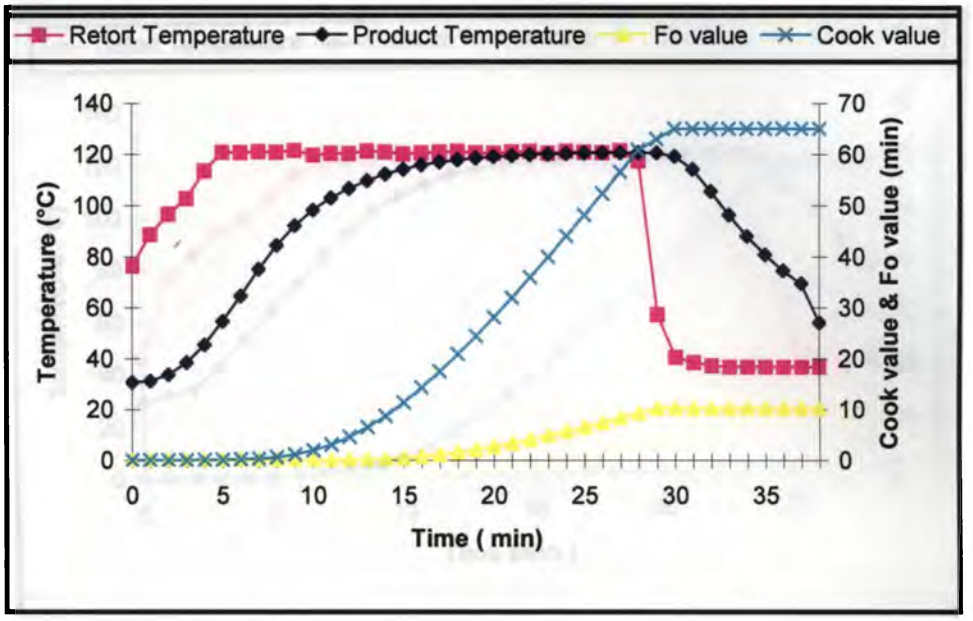


Fig 16. Heat penetration characteristics of smoked tuna in oil at 8 rpm

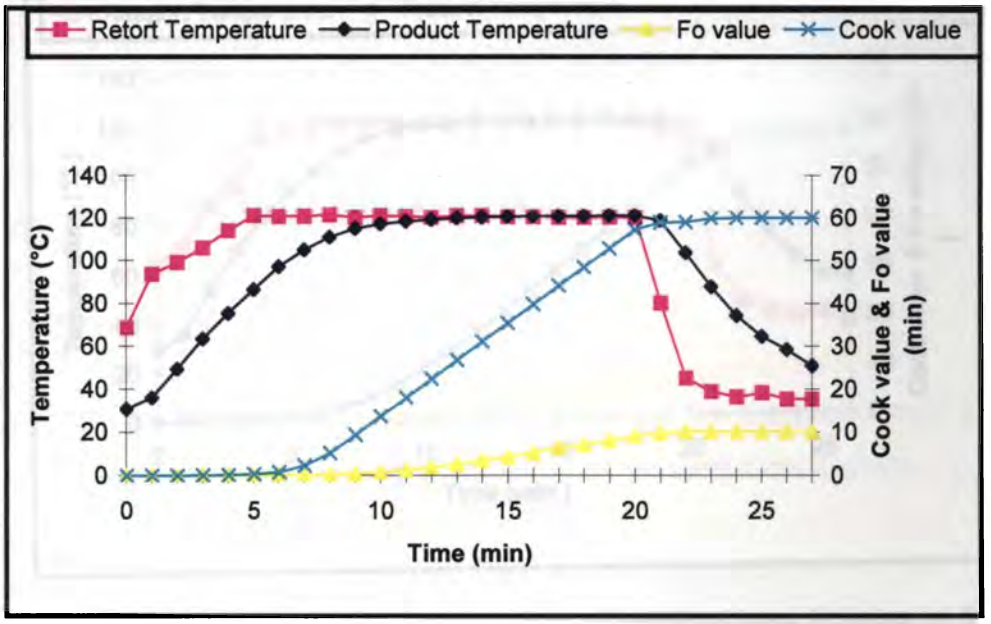


Fig 17. Heat penetration characteristics of smoked tuna in brine at 2 rpm

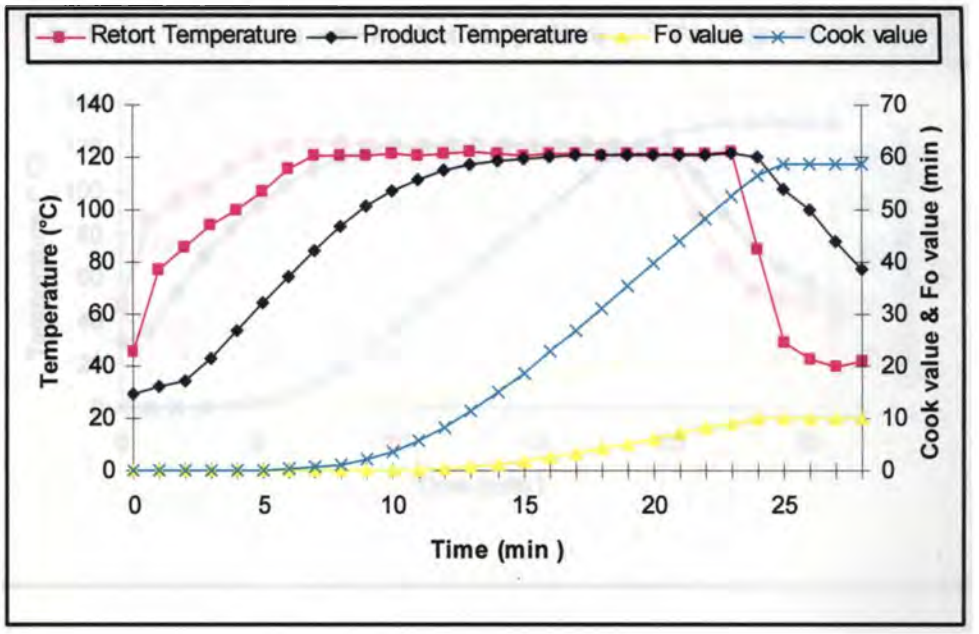


Fig 18. Heat penetration characteristics of smoked tuna in brine at 4 rpm

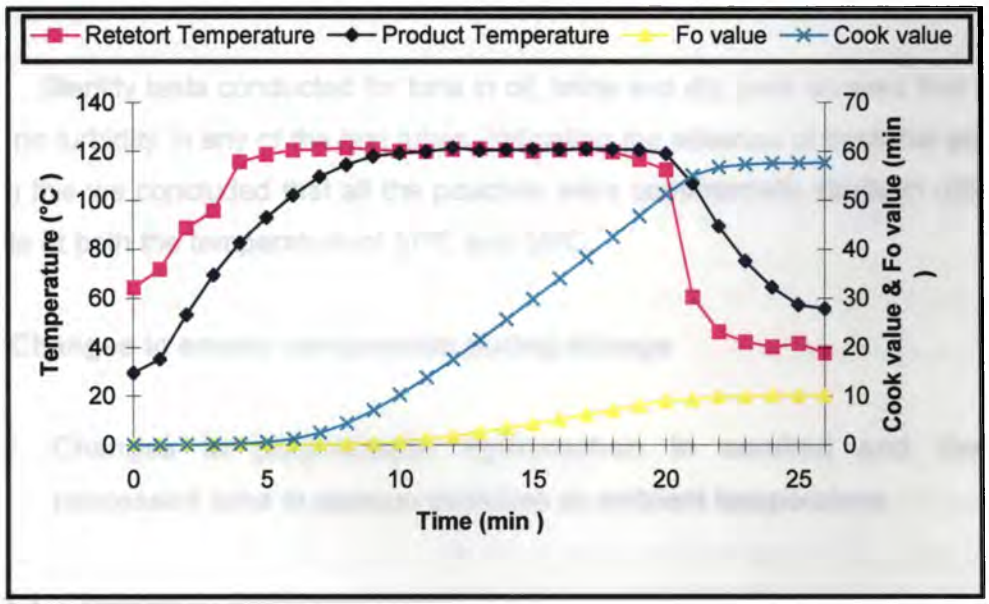


Fig .19. Heat penetration characteristics of smoked tuna in brine at 6 rpm

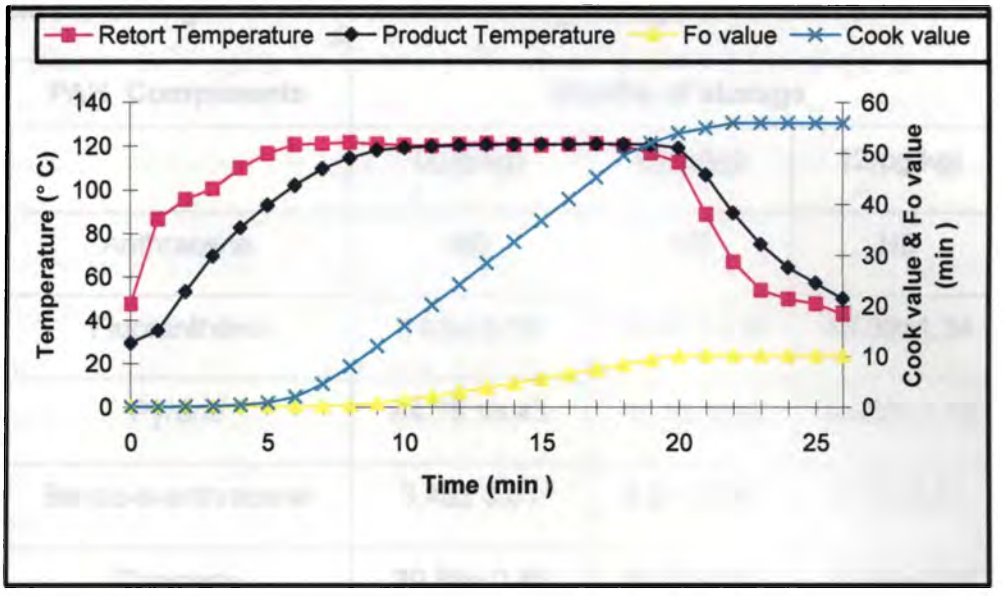


Fig. 20. Heat Penetration characteristics of smoked tuna in brine at 8 rpm

4.3.6. Sterility Tests

Sterility tests conducted for tuna in oil, brine and dry pack showed that there was no turbidity in any of the test tubes, indicating the absence of bacterial growth. From this we concluded that all the pouches were commercially sterile in different media at both the temperature of 37°C and 55°C.

4.4. Changes in smoke components during storage

4.4.1. Changes in polyaromatic hydrocarbon in smoked and thermal processed tuna in opaque pouches at ambient temperature.

4.4.1.1. Changes in PAH compounds during storage in brine

The changes in different PAH components of tuna in brine during storage in opaque pouches are given in the Table 22.

Table 22. Changes in PAH contents during storage of smoked tuna in brine

PAH Components	Months of storage		
	0(µg/kg)	6(µg/kg)	12(µg/kg)
Anthracene	ND	ND	ND
Fluoranthene	11.5± 0.15	11.78 ± 0.67	15.30±1.34
Pyrene	44.78 ±3.43	41.7± 3.50	43.23± 1.19
Benzo-a-anthracene	3.49± 0.01	3.2 ± 0.85	3.12±0.81
Chrycene	39.88± 0.45	40.21± 0.2	41.75± 0.02
Benzo-b- fluoranthene	1.5±0.71	1.18 ± 0.39	1.08±0.93
Benzo-k- fluoranthene	0.55±0.07	0.48 ± 0.11	0.35±0.49
Benzo-a-pyrene	1.65±0.05	1.40±0.01	1.39±0.09
Dibenzo-anthracene	0.81±0.01	0.80	0.79±0.02
Benzo-ghi- perylene	5.53±0.18	4.6± 0.35	4.98±0.04
Indeno pyrene	7.40±0.35	4.93±0.67	4.61±0.22

ND- Not detected.
Values are average of duplicate analysis

During storage of these thermal processed products, there was only a very slight decline in the levels of BaP. The initial values of BaP were 1.65 µg/kg which decreased slightly to 1.39 µg/kg. Since PAH compounds are basically lipophilic substances entering the food from external sources not much degradation takes places during storage. In effect this change can be due to the dilution effect of the filling medium and leaching of the compounds into it. Changes in levels of other

important polyaromatic compounds from initial to final after storage for 12 months were 0.81 to 0.79 µg/kg for dibenzo-anthracene, 3.49 to 3.12 µg/kg for benzo-a-anthracene, 1.5 to 1.08 µg/kg for benzo-b-fluoranthene, 0.55 to 0.35 µg/kg for benzo-k- fluoranthene, 7.40 to 4.61 µg/kg for indenopyrene and 44.78 to 43.23 µg/k for pyrene. The heat processing method and composition of the food play an important factor in determining the PAH content. (Yamazaki *et al.,* 1977: Larsson *et al.,* 1983). Obana *et al.,* (1984) has reported that cooking increases the PAH content in fish. This is because cooking results in loss of moisture and concentration of the solid mass. As a result we see an increase in the level. During storage there is a decrease in the compounds due to the breakdown of these compounds.

4.4.1.2. Changes in PAH compounds during storage in oil

The changes in PAH compounds after thermal processing in oil medium is given in Table 23. The BaP content of the smoked tuna after thermal processing was 1.58 µg/kg which decreased to 1.13 µg/kg after storage for 12 months. The changes from initial to final content in other PAH compounds of importance like dibenzo-anthracene was 1.25 to 1.15 µg/kg, benzo-a-anthracene 3.51 to 3.80 µg/kg, benzo-b-fluoranthene 1.93 to 1.50 µg/kg, benzo-k-fluoranthene 0.23 to 0.18µg/kg, indenopyrene 7.38 to 36.30 µg/kg and pyrene 48.43 to 34.73 µg/kg. The sunflower oil which was used as the filling medium may not have contributed to the final BaP levels. Increase in levels of indenopyrene shows that this may have been deposited in the tissue from the filling medium. Studies on canned fish in oil have shown that oils can also contribute to the PAH levels. Some oils have shown to contain levels of PAH upto 50 µg/kg (Slayne, 2003). In smoked and canned sardines, the BaP levels were up to five times more in the oil than in the fish (Lawrence &Weber, 1984).

Table 23: Changes in PAH contents during storage of smoked tuna in oil

PAH Components	Months of storage		
	0(µg/kg)	6(µg/kg)	12(µg/kg)
Anthracene	ND	ND	ND
Fluoranthene	7.70±0.21	8.50±0.21	9.80±0.81
Pyrene	48.43±2.02	44.93±3.64	34.73±7.04
Benzo-a-anthracene	3.51± 2.10	3.00±0.49	3.80±0.71
Chrysene	26.61±31.09	15.97±0.78	23.08±31.00
Benzo-b- fluoranthene	1.93±1.31	1.50±0.21	1.50±0.49
Benzo-k- fluoranthene	0.23±0.32	0.55±0.07	0.18±0.25
Benzo-a-pyrene	1.58±0.29	1.20±0.2	1.13±0.28
Dibenzo-anthracene	1.25±0.35	1.08±0.39	1.15±0.35
Benzo-GHI- perylene	4.83±3.99	5.03±0.88	4.50±2.76
Indenopyrene	7.38±1.58	15.78±0.53	36.30±16.69

ND- Not detected.
Values are average of duplicate analysis

4.4.1.3. Changes in PAH compounds during storage in dry pack

The changes in PAH levels of smoked tuna in dry pack are given in Table 24.

Table 24. Changes in PAH contents during storage of smoked tuna dry pack

PAH Components	Months of storage		
	0(µg/kg)	6(µg/kg)	12(µg/kg)
Anthracene	ND	ND	ND
Fluoranthene	18.43 ±25.98	19.98±7.95	17.80±0.21
Pyrene	2.10 ±0.00	1.35±1.90	3.25±2.76
Benzo-a-anthracene	4.06 ±13.85	3.20±4.31	3.53±1.59
Chrycene	45 ±2.52	43.15±1.91	40.02±1.32
Benzo-b- fluoranthene	2.45± 0.1	1.30±1.84	2.00±1.06
Benzo-k- fluoranthene	0.75± 0.23	0.73±0.18	0.83±0.60
Benzo-a-pyrene	1.49 ±0.28	1.45±0.14	1.40±0.18
Dibenzo-anthracene	1.95 ±2.75	1.18±0.11	1.40±0.78
Benzo-ghi- perylene	3.05 ±4.31	2.73±1.24	4.50±0.28
Indeno pyrene	7.06 ±0.05	4.35±0.14	7.43±0.04

ND- Not detected.

Values are average of duplicate analysis

There is no marked difference in the levels of PAH compounds in smoked tuna dry pack after storage for 12 months. The final content of BaP was 1.40 µg/kg, dibenzo-anthracene 1.40µg/kg, benzo-a-anthracene 3.53 µg/kg, benzo-b-fluoranthene 2.00 µg/kg, benzo-k-fluoranthene 0.83µg/kg, indenopyrene 7.43 µg/kg and pyrene 3.25 µg/kg. BaP contents are affected by environmental factors like the initial exposure to light and oxygen when the fish is just smoked. Studies by

(Simko, 2005) showed that the amount of decomposed BaP is proportionally equal to the time of light exposure. Law *et al.*, (2002) suggested that toxicity levels of the BaP content may also decrease due to the antioxidant capabilities of phenol derivatives and other compounds present in the fish. The pouches used in this study were opaque ones and there was no chance of photo degradation of BaP due to light or air exposure. The degradation in BaP that has taken place may be during the time lapse for thermal processing after smoking the tuna. Diffusion of the BaP into the interior portion may also have taken place, but samples were analysed as a composite mixture and not much change could be expected during storage.

4.4.2. Changes in total carbonyls of smoked and thermal processed tuna in different retortable pouches during storage at ambient temperature

4.4.2.1. Changes in total carbonyl values during storage in brine

Changes in total carbonyl values are given in Table 25

Table 25: Changes in total carbonyl values in different retortable pouches during storage in brine medium

Type of packaging material	Months of storage			
	0(mg/kg)	4(mg/kg)	8(mg/kg)	12(mg/kg)
INOP	0.98±0.01	0.75±0.01	0.68±0.02	0.44±0.01
INST	0.89±0.1	0.75±0.02	0.67±0.03	0.53±0.4
IMST	0.94±0.01	0.78±0.01	0.68±0.01	0.54±002
INTL	1.01±0.02	0.61 ±0.01	NA	NA

* Each value is represented by the average ± standard deviation of at least 3 determinants.
** NA Samples rejected after 4 months.

During thermal processing of smoked tuna in brine the levels of total carbonyls have shown a decrease from 0.98 to 0.44 mg/kg in INOP, 0.89 to 0.53 mg/kg in INST, 0.94 to 0.54 mg/kg in IMST and from 1.01 to 0.61 mg/kg in INTL pouches. This decrease in the levels of carbonyls may be due to the degradation of carbonyls to different compounds. The brown coloured compounds produced due to the reactions may have leached out into the filling medium during thermal processing along with the moisture that is expelled from the tuna meat. Gu *et al.*, (2001) suggested that the browning of the liquid medium may be due to the amino-carbonyl reactions occurring during thermal processing and subsequent storage, due to the leaching out of the non-protein nitrogen and free sugars out of the meat into the filling media.

4.4.2.2. Changes in total carbonyl values during storage in oil

The total carbonyl content values for tuna in oil are given in Table 26

Table 26: Changes in total carbonyl values of tuna in different retortable pouches during storage in oil medium

Type of packaging material	Months of storage			
	0(mg/kg)	4(mg/kg)	8(mg/kg)	12(mg/kg)
INOP	0.93±0.02	0.70± 0.02	0.61±0.01	0.58±0.02
INST	0.92±0.01	0.65 ±0.01	0.56±0.06	0.64±0.05
IMST	0.95±0.03	0.70±0.01	0.76±0.05	0.63±0.03
INTL	0.94±0.01	0.67±0.03	NA	NA

* Each value is represented by the average ± standard deviation of at least 3 determinants.

** NA Samples rejected after 4 months.

The initial carbonyl values of tuna packed in oil medium were 0.93 mg /kg for opaque pouches, 0.92 mg/kg in indigenous see through pouches, and 0.95 mg /kg for imported see through pouches and 0.94 mg/kg in two layered pouches. The final values were 0.58 mg/kg during storage in INOP for 12 months. The values decreased to 0.63 mg/kg and 0.64 mg/kg for IMST and INST. The carbonyl content was 0.67mg/kg in INTL after storage for 4 months respectively.

4.4.2.3. Changes in total carbonyl values during storage in dry pack

The changes in total carbonyls of smoked tuna as dry pack are given in Table 27.

Table 27: Changes in total carbonyl values in different retortable pouches during storage as dry pack.

Type of packaging material	Months			
	0(mg/kg)	4(mg/kg)	8(mg/kg)	12(mg/kg)
INOP	0.85±0.01	0.71±0.01	0.54±0.02	0.44±0.02
INST	0.80±0.02	0.75±0.06	0.68±0.02	0.66±0.01
IMST	0.81±0.01	0.72±0.05	0.66±0.01	0.63±0.03
INTL	0.85±0.02	0.75±0.01	NA	NA

*Each value is represented by the average ± standard deviation of at least 3 determinants

** NA Samples rejected after 4 months.

The initial values of carbonyls in tuna packed in dry pack after heat sterilization were 0.85, 0.81, 0.80 and 0.85mg/kg for opaque, see through

indigenous, see through opaque and two layered pouches respectively. The final levels of carbonyls after storage for twelve months were 0.44, 0.66 and 0.63mg/kg for INOP, INST and IMST pouches. In two layer pouches the value decreased to 0.75 mg/kg after 4 months of storage. This decrease is mainly due to the breakdown of carbonyl compounds into other compounds due to the browning reactions.

Various media had a different effect on the carbonyl content. Further when Tukey's test was performed to analyse the effect of oil, brine and no filling medium (dry) it was found that dry pack and oil gave the same effect. In the brine medium the values were significantly less ($p \leq .01$). when the variation of carbonyl content in different pouches were examined it was found that the maximum content was in tuna packed in Indigenous see through pouches and the minimum content in Indigenous opaque pouches and varied statistically different for all pouches ($p \leq .01$). Carbonyl content was found to be decreasing significantly during the storage period ($p \leq .01$).

4.4.3. Changes in total phenols of smoked and thermal processed tuna in different retortable pouches during storage at ambient temperature

4.4.3.1. Changes in phenol content of tuna in brine in different retortable pouches.

The changes in the phenol content during storage of smoked tuna in brine after thermal processing and during storage are given in Table 28. The initial values for opaque, indigenous see through, imported see through and two layered pouches were 0.80, 0.72, 0.83 and 0.76 mg/kg respectively. It was observed that during the storage period of twelve months there was a decrease in the phenol content to 0.22, 0.30 and 0.39mg/kg in INOP, IMST and INST pouches respectively. The phenol content decreased to 0.64 mg/kg after 4 months of storage in INTL pouches. The decrease may be due to the covalent binding of the

quinones, formed from the oxidized phenols to the proteins. Della *et al.*, (1996) has reported that phenolic compounds generally do not undergo any significant change during thermal stress. The antioxidant compounds present in the smoked fish will help in retarding the oxidation of lipid in the fish as indicated by the relatively low TBA values in tuna packed in brine medium

Table 28: Changes in total phenol content of tuna in brine in different retortable pouches

Type of packaging material	Months of storage			
	0(mg/kg)	4(mg/kg)	8(mg/kg)	12(mg/kg)
INOP	0.80± 0.02	0.69±0.01	0.54± 0.02	0.22±0.01
INST	0.72±0.01	0.56±0.11	0.35±0.02	0.30±0.01
IMST	0.83±0.01	0.64±0.02	0.41±0.01	0.39±0.04
INTL	0.76±0.02	0.64±0.01	NA	NA

*Each value is represented by the average ± standard deviation of at least 3 determinants.

** NA Samples rejected after 4 months.

4.4.3.2. Changes in total phenol content of tuna in oil in different retortable pouches.

The changes in phenol content of smoked tuna in oil medium are given in Table 29. The phenol content decreased from 0.85 to 0.55 mg/kg in INOP pouches, 0.86 to 0.38 mg/kg in INST pouches and from 0.88 to 0.42 mg/kg in IMST pouches after storage for 12 months. In the case of INTL pouches the values reduced from 0.89 to 0.72 mg/kg after storage for 4 months. These results are in agreement with the finding for tuna packed with extra virgin olive oil which contained high quantities of antioxidants and their value was found to be decreasing after sterilization and storage (Aubourg *et al.*, 1998). This loss in the

phenols may be due to the migration into the aqueous filling media or due to the interactions with the fish muscle (Waters *et al.*, 1994). The recovery of phenol compounds vary in lipid and water systems. Working on model systems Issenberg *et al.*, (1971) found that for certain phenols the percentage of recovery was different in water and lipid systems, whereas for some it was same in both systems. Thus the total phenols recovered depend on the levels of individual phenol and percentage of fat in the food (Maga, 1988). The data on phenols correlated well with the rancidity factors like TBA and FFA, where the overall values were low and slow increasing trend was observed in three mediums used in the study.

Table 29: Changes in total phenol content during storage of tuna in oil in different retortable pouches

Type of packaging material	Months of storage			
	0(mg/kg)	4(mg/kg)	8(mg/kg)	12(mg/kg)
INOP	0.85±0.01	0.78±0.03	0.67±0.01	0.55±0.04
INST	0.86±0.02	0.73±0.01	0.56±0.03	0.38±0.02
IMST	0.88±.01	0.64±0.01	0.47±.018	0.42±0.02
INTL	0.89±0.01	0.72 ±0.02	NA**	NA**

* Each value is represented by the average ± standard deviation of at least 3 determinants.

** NA Samples rejected after 4 months based on sensory.

4.4.3.3. Changes in total phenol for smoked and thermal processed tuna in dry pack in different retortable pouches.

The values of smoked tuna in dry pack are given in Table 30. Initial total phenol values for INOP, IMST, INST and INTL were 0.78, 0.70, 0.86, and 0.61 mg/kg, respectively. These values showed a decreasing trend throughout the period of storage and final levels were 0.21, 0. 20 and 0.15 mg/kg after 12 months of storage packed in first three pouches. The levels of phenols in INTL pouches decreased to 0.40 mg/kg after four months of storage. This decreasing trend may be due to the interaction between the proteins and phenols when high levels of phenol react with the polar compounds from the tuna muscles to form new complexes (Waters *et al.*, 1994).

Table 30. Changes in total phenol during storage of smoked tuna in dry pack in different retortable pouches.

Type of packaging material	Months of storage			
	0(mg/kg)	4(mg/kg)	8(mg/kg)	12(mg/kg)
INOP	0.78±0.013	0.61±0.01	0.36±0.02	0.25±0.01
INST	0.70±0.02	0.57±0.05	0.35 ± .04	0.20±0.01
IMST	0.86±0.01	0.57±0.11	0.35±0.01	0.15±.06
INTL	0.61±0.01	0.40±0.01	NA	NA

Each value is represented by the average ± standard deviation of at least 3 determinants.

** NA Samples rejected after 4 months

The phenol content also varied significantly for the three different medium. When the Tukey's test was performed to analyse the extent of variation among the pouches, it was found that the phenol values were significantly different for the entire three medium ($p \leq 0.01$). Effect of different pouches on the phenol content was also significantly different from each other. Minimum content of phenol was found in tuna packed in imported see through pouches and maximum in indigenous opaque pouches which was also statistically significant from other pouches ($p \leq 0.01$).Phenol content was found to be decreasing significantly during the storage period ($p \leq 0.01$).

4.5. Changes in biochemical components during storage of smoked and thermal processed tuna in brine, oil and dry pack.

4.5.1. Changes in total amino acid content of smoked and thermal processed tuna during storage at ambient temperature (28±1°C) in opaque pouches.

The changes in total amino acid content of tuna during storage in brine are given in Table 31. No significant decrease or loss of amino acids was observed during storage of thermal processed tuna. For tuna packed in brine initial and final content of asparic acid was 9.14 and 11.29 %, alanine 8.77 and 10.01%, valene 7.45 and 9.11%, cysteine 0.28 to 0.33%, isoleucine 5.49 to 7.00 %, leucine 9.46 to 10.17%, tyrosine1.45 and 1.60 %, lysine 1.72 to 1.94 % and phenylalanine 3.69 and 4.09 %. These amino acids were found increasing during storage. The changes during storage of tuna in oil are given in Table 32 and dry pack in Table 33. For tuna packed in oil medium aspartic acid content increased from 10.02 to 10.60%, valene 6.74 to 8.64 %, alanine 9.04 to 9.26%, isoleucine 5.32 to 6.48%, leucine 9.32 to 9.91%, phenylalanine 3.54 to 3.81, lysine 1.78 to 1.48 % and tryptophan 1.14 to 1.48 %. In smoked tuna dry pack the increase in the following amino acids like valene 6.64 to 8.67 %, methionine 1.56 to 2.34 %, isoleucine 5.22

to 6.55 %, leucine 9.20 to 9.91 %, lysine 1.62 to 1.90 % and phenylalanine 3.55 to 3.94 % showed an increase after 12 months.

Arginine, lysine and leucine are the major essential amino acids in aquatic organisms (Rosa & Nunes, 2004.) The works of Opstvedt *et al.*, (1984) found that roasting of meat and fish at 110-140°C had no effect on the total amino acid content. Major nutritive loss during processing is deterioration of the protein quality due to the non-enzymatic browning or maillard browning. Lysine is an essential amino acid present in the lowest amount. The presence of ε- amino group makes it a very reactive compound. Krylova *et al.*, (1970) and Kako, (1968) concluded that the smoke-associated carbonyl compounds react with protein amino groups whereas phenols primarily react with sulfhydryl groups.

Seet and Brown, (1983) and Castrillon *et al.*, (1996) reported no increase in the amino acid content of canned tuna using water as the filling medium. Similar findings have been reported by Mohan *et al.*, (2006) in prawn kuruma. Some of the heat-labile amino acids like cysteine increased after processing and this can be related to the formation of disulfide linkages during retorting. Castrillon *et al.*, (1996) observed loss of histidine to be comparable with that of cysteine and also found threonine and leucine decrease by less than 1%. During storage certain amino acids were found to increase while some decreased. This is mainly because of the variations in individual amino acid content or their diffusion into the filling medium. However the total percentage of amino acids remains the same.

Table 31: Changes in amino acids during storage of smoked and thermal processed tuna in brine

	Months of storage			
Amino acid	**0 (%)**	**4 (%)**	**8 (%)**	**12 (%)**
Aspartic acid	9.14	9.51	10.72	11.29
Threonine	5.085	5.78	4.76	5.07
Serine	6.52	6.54	5.91	6.30
Glutamic acid	13.48	13.87	14.08	10.43
Proline	0.63	0.72	0.71	0.63
Glycine	8.22	7.87	7.36	7.86
Alanine	8.77	9.09	8.80	10.01
Valene	7.45	6.88	8.04	9.11
Cystene	0.28	0.43	0.35	0.33
Methionine	2.48	2.12	1.71	2.52
Iso leucine	5.49	5.28	6.21	7.00
Leucine	9.46	9.66	9.40	10.17
Tyrosine	1.45	1.70	1.49	1.60
Phenylalanine	3.69	3.57	3.72	4.09
Histidine	8.72	5.37	7.66	5.97
Lysine	1.72	1.94	1.55	1.94
Arginine	2.20	3.58	1.84	1.01
Tryptophan	1.25	1.56	1.79	1.26

Table 32: Changes in amino acids during storage of smoked and thermal processed tuna in oil

	Months of storage			
Amino acid	**0 (%)**	**4 (%)**	**8 (%)**	**12 (%)**
Aspartic acid	10.02	9.44	10.10	10.60
Threonine	5.30	5.36	5.52	4.46
Serine	5.97	6.25	8.50	4.98
Glutamic acid	14.39	13.75	13.54	14.4
Proline	0.66	0.82	0.82	0.88
Glycine	8.46	8.11	8.62	8.19
Alanine	9.04	9.03	9.48	9.26
Valene	6.74	6.87	8.43	8.64
Cystene	0.52	0.48	0.32	0.40
Methionine	1.18	2.07	1.40	2.06
Iso leucine	5.32	5.80	6.58	6.48
Leucine	9.32	9.58	10.10	9.91
Tyrosine	1.52	1.76	1.53	1.24
Phenylalanine	3.54	3.87	0.75	3.81
Histidine	7.71	6.86	7.40	7.37
Lysine	1.78	1.63	1.88	1.84
Arginine	3.41	2.26	0.85	0.84
Tryptophan	1.14	1.25	1.46	1.48

Table 33. Changes in amino acids during storage of smoked and thermal processed tuna dry pack

	Months of storage			
Amino acid	**0 (%)**	**4 (%)**	**8 (%)**	**12 (%)**
Aspartic acid	10.67	9.72	9.06	9.68
Threonine	5.13	5.29	4.85	4.48
Serine	6.65	6.76	6.98	4.59
Glutamic acid	14.21	13.69	13.85	13.31
Proline	0.70	0.68	0.79	0.73
Glycine	8.65	8.67	8.33	9.11
Alanine	9.22	9.08	9.14	9.29
Valene	6.64	6.54	8.16	8.67
Cystene	0.42	0.38	0.43	0.42
Methionine	1.56	1.75	1.67	2.34
Iso leucine	5.22	4.91	6.30	6.55
Leucine	9.20	9.15	9.57	9.91
Tyrosine	1.46	1.54	1.53	1.31
Phenylalanine	3.55	3.53	3.71	3.94
Histidine	7.51	8.13	8.30	7.92
Lysine	1.62	1.82	0.73	1.90
Arginine	2.49	3.39	1.63	1.25
Tryptophan	1.55	1.14	1.25	1.55

4.5.2. Changes in biogenic amine content of smoked and thermal processed tuna packed in indigenous opaque pouches

Temperature abuse before thermal processing can cause histamine fish poisoning in commercially canned fish products (Taylor, 1983). Hence histamine and related amines like putrescine and cadaverine are of utmost importance in determining the final quality of the product.

4.5.2.1. Changes in biogenic amines during storage in brine

The different changes in levels of biogenic amines are given in Table 34.

Table 34. Changes in biogenic amines of smoked and thermal processed tuna in brine

	Months of storage		
Biogenic amines	**0(ppm)**	**6(ppm)**	**12(ppm)**
Putrescine	0.38±0.09	0.25±0.02	0.31±0.06
Cadaverine	0.26±0.05	0.26±0.01	0.21±0.01
Spermidine	0.17±0.07	0.07±0.03	0.11±0.01
Spermine	0.17±0.07	0.12±0.02	0.13±0.01
Tyramine	0.27±0.04	0.19 ±0.01	0.17±0.01
Agmatine	0.21±0.01	0.19 ±0.01	0.12±0.04
Histamine	7.13±0.02	4.93 ±0.01	4.72±0.29

* Each value is represented by the average ± standard deviation of at least 3 determinants.

The histamine levels of smoked tuna in brine decreased from 7.13 ppm to 4.72 ppm after 12 months of storage. The initial and final level of putrescine was 0.38 and 0.31 ppm ; cadaverine 0.26 and 21 ppm; spermidine 0.17 and 0.11 ppm; tyramine 0.27 and 0.17 ppm and agmatine 0.21 and 0.12 ppm. The levels of spermidine remained the same after thermal processing whereas the spermine, agmatine and tyramine levels increased slightly after thermal processing. Since thermal processing brings about complete destruction of the bacteria, there is no significant change during storage. The only changes that take place may be due to the dilution into the filling medium, thereby showing lower levels after storage .

4.5.2.2. Changes in biogenic amines during storage in oil

Changes in biogenic amine content of tuna in oil are given in Table 35.

Table 35. Changes in biogenic amines of smoked and thermal processed tuna in oil

	Months of storage		
Biogenic amines	**0(ppm)**	**6(ppm)**	**12(ppm)**
Putrescine	0.68±0.05	0.24±0.01	0.58± 0.11
Cadaverine	0.79±0.02	0.08±0.03	0.64±0.25
Spermidine	0.11±0.04	0.15±0.20	0.13±0.02
Spermine	0.15±0.01	0.13±0.01	0.11±0.04
Tyramine	0.12±0.04	0.18±0.05	0.11±0.02
Agmatine	0.47±0.26	0.24±0.03	0.13±0.06
Histamine	6.93±0.41	5.87±1.09	4.93±0.35

* Each value is represented by the average ± standard deviation of at least 3 determinants.

The level of histamine was 6.93 ppm after thermal processing. It was observed that values decreased to 4.93 ppm during the storage period of 12 months. The final levels of different biogenic amines after twelve months storage are putrescine (0.58 ppm), cadaverine (0.64ppm), spermine (0.11 ppm), spermidine (0.13ppm), tyramine (0.11ppm) and agmatine (0.13ppm). The decrease in the levels of biogenic amines can be attributed to the leaching out of the same into the oil medium during long time storage. The studies of Durr *et al.*, (1980); Shalaby *et al.*, (1990) have shown that the biogenic amine content of fish is not significantly reduced by sterilization and storage process, though some histamine may be partially lost which supports our findings.

4.5.2.3. Changes in biogenic amines during storage in dry pack

The changes in biogenic amines of smoked tuna dry pack are given in Table 36.

Table 36. Changes in biogenic amines of smoked and thermal processed tuna dry pack

Biogenic amines	Months of storage		
	0 (ppm)	6 (ppm)	12 (ppm)
Putrescence	0.24±0.02	0.16±0.03	0.17±0.02
Cadaverine	0.27±0.02	0.2±0.01	0.2±0.01
Spermidine	0.17±0.00	0.33±0.05	0.11±0.03
Spermine	0.24±0.00	0. 19±0.01	0.17±0.01
Tyramine	0.20±0.08	0.21±0.14	0.19±0.11
Agmatine	0.24±0.11	0.23±0.04	0.19±0.03
Histamine	6.50±0.57	6.23±0.26	6.43±0.34

* Each value is represented by the average ± SD of at least 3 determinants.

It is seen that the final levels of histamine in the tuna dry pack was 6.43 ppm which is well below the 50 ppm recommended by FDA, 1995. The levels of other biogenic amines like putrescine, cadaverine, spermidine, spermine, tyramine and agmatine were 0.17, 0.2, 0.11, 0.17, 0.19 and 0.19 ppm respectively.

The present study also has demonstrated that there is no significant change in the levels of biogenic amines during sterilization and subsequent storage. This is mainly due to the fact that once the amines are formed they do not undergo any further change during hot smoking even though the histamine developing bacteria is eliminated (Bremer *et al.*,(1998). The histamine content of the canned fish is mainly affected by the freshness of fish, fish species, and chilling conditions during transportation and precooking methods (Tuan and Tsai, 1981). Luten *et al.*, (1992) observed that 90% of the biogenic amines could be recovered from tuna after sterilization and hence there is no significant change in their content after sterilization.

4.6. Shelf life studies

Shelf life of the smoked and thermal processed tuna was evaluated by various biochemical, textural and sensory methods by studying the changes during storage at accelerated and ambient temperature. Tuna packed in INOP, IMST, and INST pouches were found to have a shelf life of more than a year at both temperatures sensorily. However samples packed in INTL pouches were rejected after 2 months for accelerated temperature and 4 months at ambient temperature in all the three types of packs studied.

4.6.1. Changes in pH values of smoked and thermal processed tuna at ambient (28 ± 2°C) and accelerated shelf life (37 ± 2°C)

4.6.1.1. Changes in pH values during storage in brine

Changes in pH during storage of smoked tuna thermal processed in brine medium at ambient and accelerated temperature showed a decreasing trend in the pH in all the four different pouches used in this study (Figures 21-22). During ambient storage for twelve months the initial and final values for tuna packed in INOP was 6.01 and 5.60, IMST 6.00 and 5.64, INST 6.00 and 5.62. Samples packed in INTL pouches had a pH of 5.70 after 4 months of storage. In the case of accelerated storage there was no significant variation compared to that of ambient temperature stored products. The final values were 5.63 for INOP, 5.63 for IMST, 5.64 for INST and 5.72 for INTL. No major change in the pH levels is expected since pretreatment with salt and subsequent smoking reduced the moisture content and inhibited microbial growth and retarded spoilage. Leroi and Joffraud., (2000a) have found that salt has a linear decreasing effect on the pH of the fish.

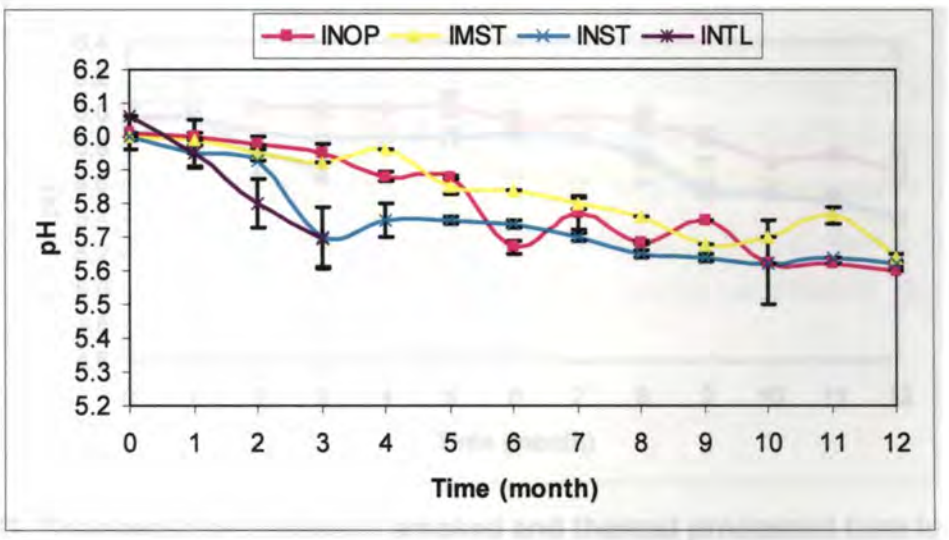


Fig. 21. Changes in pH values of smoked and thermal processed tuna in brine in different pouches during storage at 28 ± 2°C.

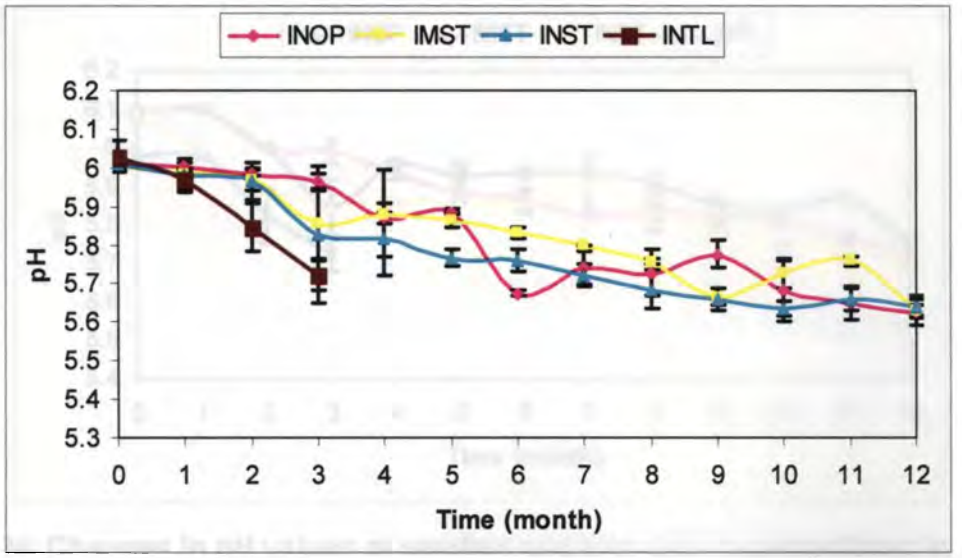


Fig. 22. Changes in pH values of smoked and thermal processed tuna in brine in different pouches during storage at 37 ± 2°C.

4.6.1.2. Changes in pH values during storage in oil

The changes in pH for smoked tuna in oil are given in Figures 23-24.

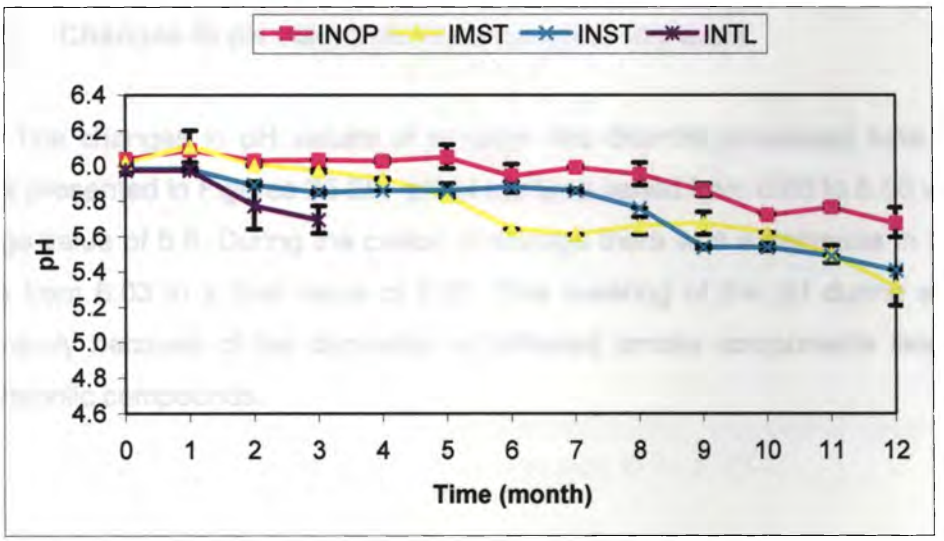


Fig. 23. Changes in pH values of smoked and thermal processed tuna in oil in different pouches during storage at 28 ± 2°C.

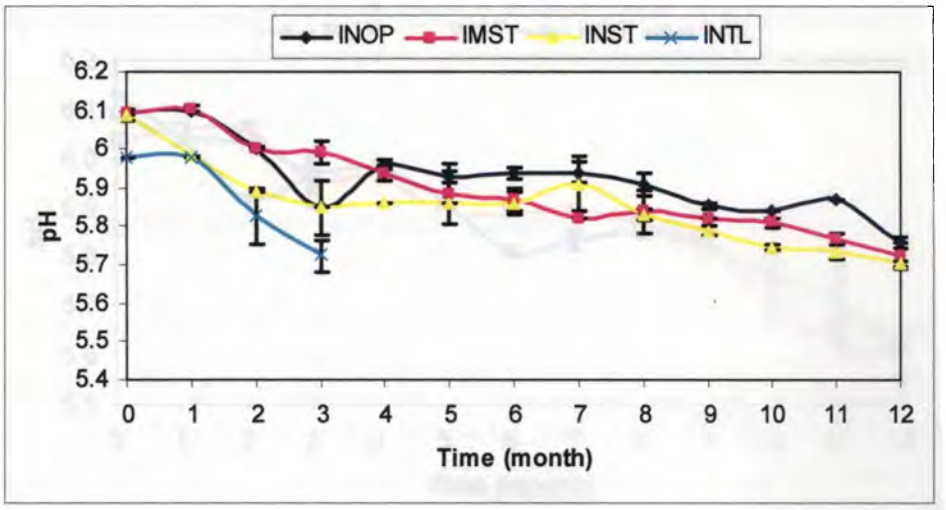


Fig. 24. Changes in pH values of smoked and thermal processed tuna in oil in different pouches during storage at 37 ± 2°C.

The final pH levels were 5.6 for samples packed in INOP, 5.62 for INST, 5.64 for IMST and 5.70 for INTL. In the case of accelerated storage the final values were similar to that of ambient temperature stored tuna. The levels were 5.63 for INOP, 5.62 for IMST, 5.64 for INST and 5.70 for INTL.

4.6.1.3. Changes in pH values during storage in dry pack

The changes in pH values of smoked and thermal processed tuna in dry pack is presented in Figures 25-26. pH of the tuna varied from 6.00 to 5.50 with an average value of 5.8. During the period of storage there was a decrease in the pH values from 6.03 to a final value of 5.50. This lowering of the pH during storage was mainly because of the deposition of different smoke components like acids and phenolic compounds.

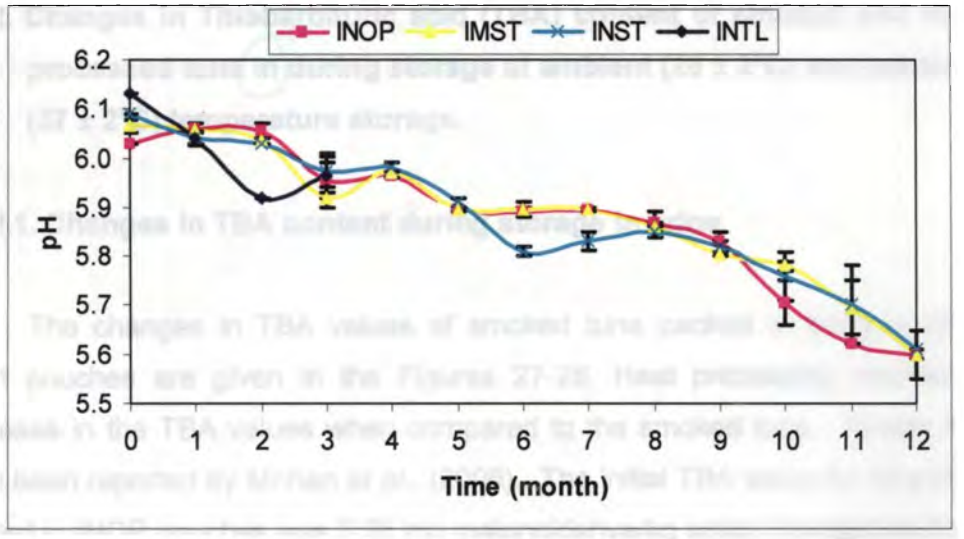


Fig. 25. Changes in pH values of smoked and thermal processed tuna in dry pack in different pouches during storage at 28 ± 2°C.

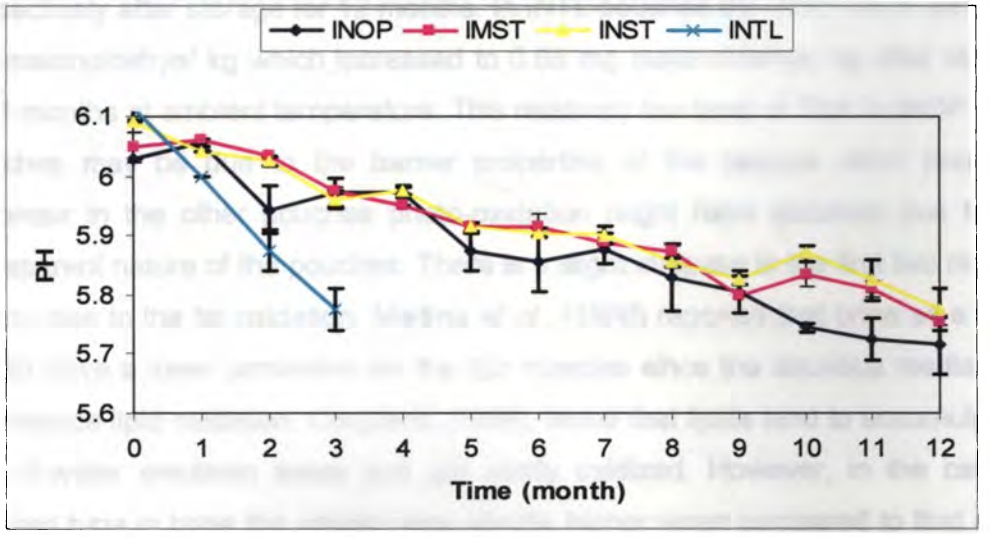


Fig. 26. Changes in pH values of smoked and thermal processed tuna in dry pack in different pouches during storage at 37 ± 2°C.

4.6.2. Changes in Thiobarbituric acid (TBA) content of smoked and thermal processed tuna in during storage at ambient (28 ± 2°C) and accelerated (37 ± 2°C) temperature storage.

4.6.2.1. Changes in TBA content during storage in brine

The changes in TBA values of smoked tuna packed in brine in different retort pouches are given in the Figures 27-28. Heat processing resulted in a decrease in the TBA values when compared to the smoked tuna. Similar finding have been reported by Mohan *et al.*, (2006). The initial TBA value for tuna in brine packed in INOP pouches was 0.26 mg malonoldehye/kg which changed to 0.18 mg malonoldehye/kg after storage for 12 months. In case of INST and IMST pouches the values increased from 0.32 mg malonoldehye/kg and 0.12 mg malonoldehye/kg to 0.33 mg malonoldehye/kg and 0.22 mg malonoldehye/kg respectively after storage for 12 months. In INTL pouches the initial value was 0.13 mg malonoldehye/ kg which increased to 0.68 mg malonoldehye/ kg after storage for 4 months at ambient temperature. This relatively low level of TBA in INOP retort pouches may be due to the barrier properties of the opaque retort pouches. Whereas in the other pouches photo-oxidation might have occurred due to the transparent nature of the pouches. There is a slight increase in the first two months mainly due to the fat oxidation. Medina *et al.,* (1998) reported that brine as a filling media have a lower protection on the fish muscles since the aqueous media tend to enhance lipid oxidation. Coupland (1996), found that lipids tend to accumulate in the oil-water emulsion areas and get easily oxidized. However, in the case of smoked tuna in brine the values were slightly higher when compared to that of oil. Aubourg & Ugliano, (2002) ; Connell, (1990) and Davis *et al.*, (1993) reported that salt content in fish muscle enhances oxidation of the unsaturated lipids.

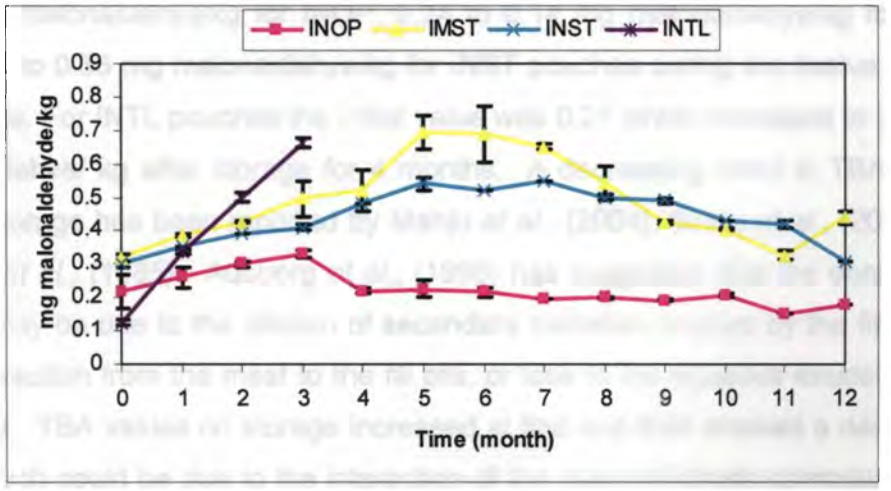


Fig. 27. Changes in TBA values of smoked and thermal processed tuna in brine in different pouches during storage at 28 ± 2°C.

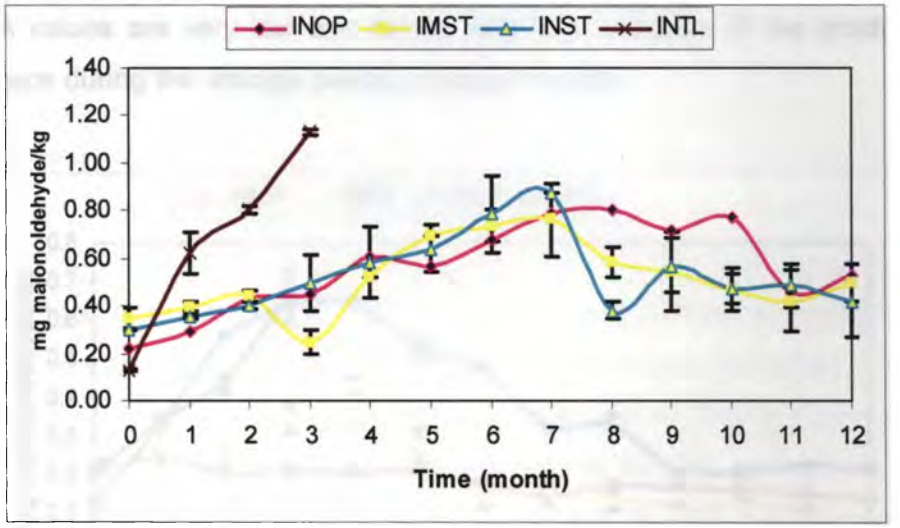


Fig. 28. Changes in TBA values of smoked and thermal processed tuna in brine in different pouches during storage at 37 ± 2°C.

4.6.2.2. Changes in TBA content during storage in oil.

Figures 29-30 give the values for changes in TBA value for smoked tuna in oil medium. The changes in TBA values ranged from initial values of 0.26 mg to

0.15 mg malonaldehye/kg for INOP, 0.24 to 0.14 mg malonaldehye/kg for IMST and 0.13 to 0.33 mg malonaldehye/kg for INST pouches during the twelve months of storage. For INTL pouches the initial value was 0.21 which increased to 0.61 mg malonaldehye/ kg after storage for 4 months. A decreasing trend in TBA values during storage has been reported by Manju *et al.*, (2004), Bindu *et al.*, (2007) and Tanaka *et al.*, (1985). Aubuorg *et al.*, (1995) has suggested that the decrease in values may be due to the dilution of secondary oxidation product by the fill oils, or their extraction from the meat to the fill oils, or loss to the aqueous exudates from the meat. TBA values on storage increased at first and then showed a decreasing trend which could be due to the interaction of the malonaldehyde compounds with proteins, amino acid, etc. which can cause its reduction (Fernandez *et al.,* 1997 and Gomez *et al.,* 2003). The limit for TBA is 1-2 mg malonaldehyde/kg after which the fish will develop a rancid odour (Connell, 1990). Our studies have shown that the TBA values are very low and hence very little oxidation of the products has taken place during the storage period of twelve months.

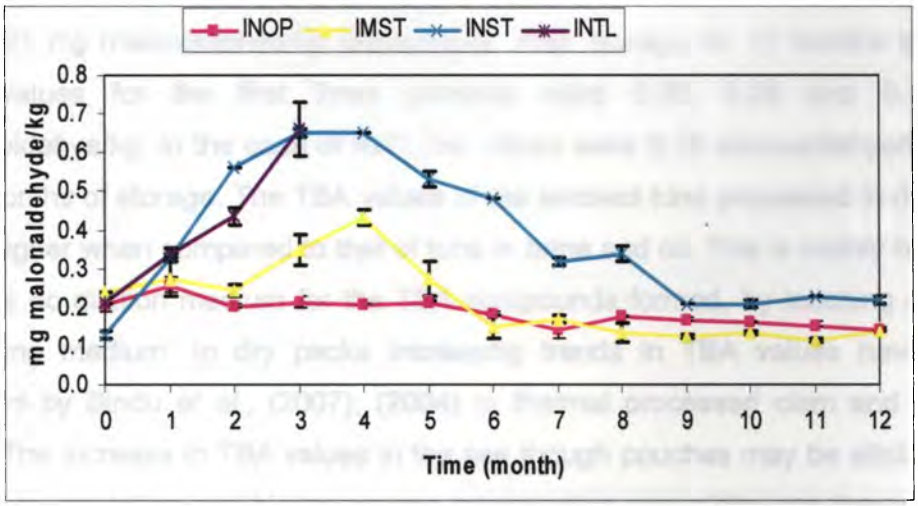


Fig. 29. Changes in TBA values of smoked and thermal processed tuna in oil in different pouches during storage at 28 ± 2°C.

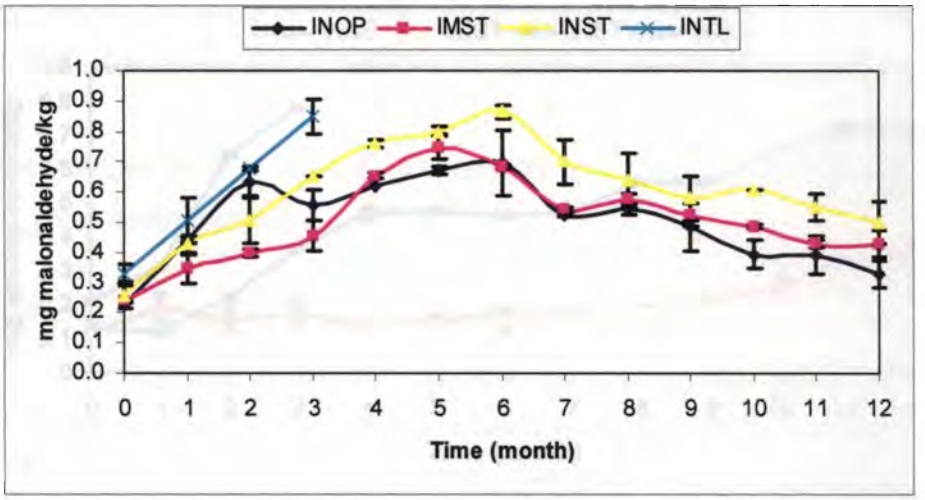


Fig. 30. Changes in TBA values of smoked and thermal processed tuna in oil in different pouches during storage at 37 ± 2°C.

4.6.2.3. Changes in TBA content during storage in dry pack

The TBA values of smoked tuna packed in dry pack are given in figures 31-32. The initial values for INOP, IMST, INST and INTL were 0.12 mg, 0.24, 0.13 and 0.21 mg malonoldehye/kg respectively. After storage for 12 months the final TBA values for the first three pouches were 0.36, 0.28 and 0.71 mg malonoldehye/kg. In the case of INTL the values were 0.78 malonaldehye/kg after four months of storage. The TBA values of the smoked tuna processed in dry pack were higher when compared to that of tuna in brine and oil. This is mainly because there is no dilution medium for the TBA compounds formed, by leaching out into the filling medium. In dry packs increasing trends in TBA values have been reported by Bindu *et al.*, (2007); (2004) in thermal processed clam and mussel meat. The increase in TBA values in the see though pouches may be attributed to the photo- oxidation and higher oxygen transmission rate of the see through INTL pouches.

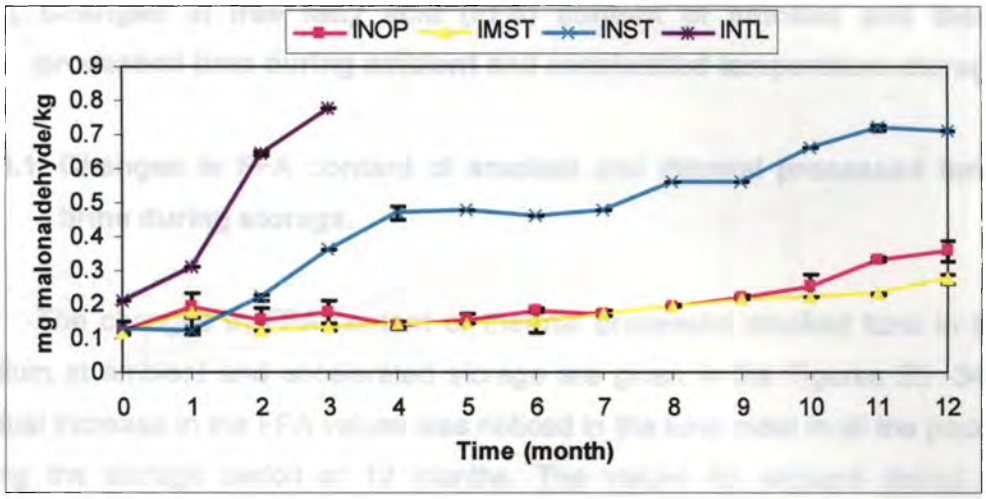


Fig. 31. Changes in TBA values of smoked and thermal processed tuna in dry pack in different pouches during storage at 28 ± 2°C.

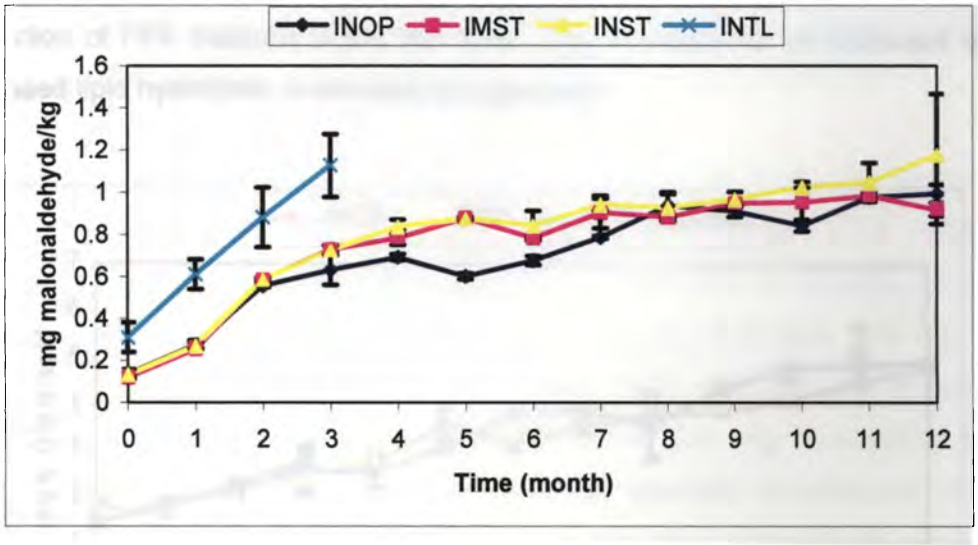


Fig. 32. Changes in TBA values of smoked and thermal processed tuna in dry pack in different pouches during storage at 37 ± 2°C.

4.6.3. Changes in free fatty acid (FFA) content of smoked and thermal processed tuna during ambient and accelerated temperature storage.

4.6.3.1. Changes in FFA content of smoked and thermal processed tuna in brine during storage.

The changes in FFA content of thermal processed smoked tuna in brine medium at ambient and accelerated storage are given in the Figures 33 -34. A gradual increase in the FFA values was noticed in the tuna meat in all the pouches during the storage period of 12 months. The values for ambient stored tuna increased from 1.88 to 4.80 mg % in INOP pouches, 1.94 to 5.96 mg % in INST pouches and 1.42 to 4.94 mg% in IMST pouches. For INTL layered pouches there is an increase from 1.46 to 2.82 mg% after storage period of four months. Aubourg *et al.*, (1990) and Aubourg *et al.*, (1997a) have reported that canning increases the proportion of FFA fractions in the fish meat. The increase can be attributed to an increased lipid hydrolysis at elevated temperatures.

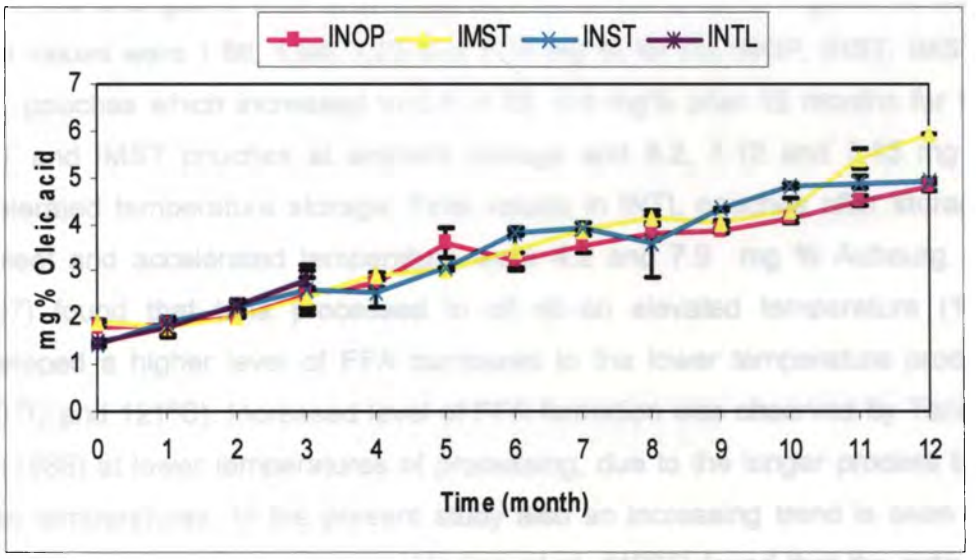


Fig. 33. Changes in FFA values of smoked and thermal processed tuna in brine in different pouches during storage at 28 ± 2°C.

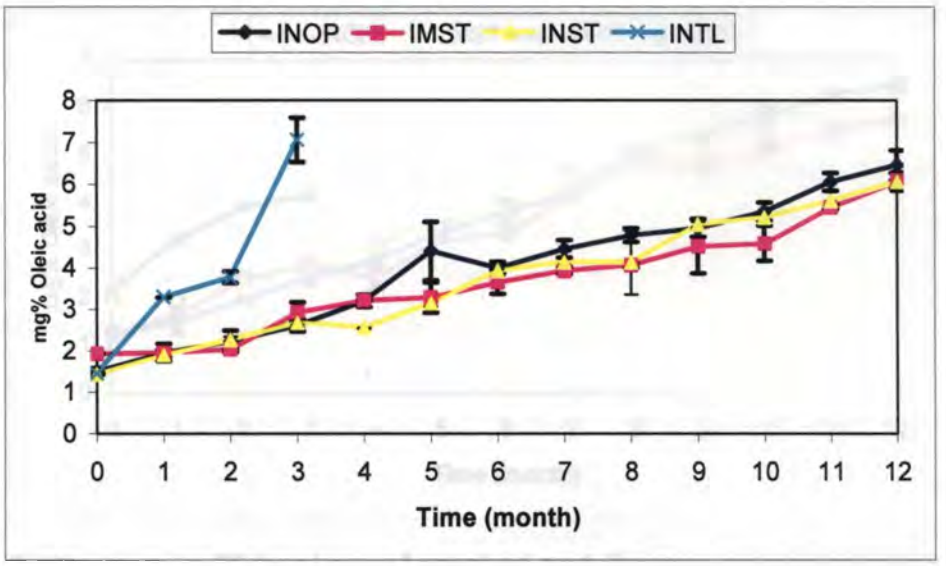


Fig. 34. Changes in FFA values of smoked and thermal processed tuna in brine in different pouches during storage at 37 ± 2°C.

4.6.3.2. Changes in FFA content of smoked and thermal processed tuna in oil during storage.

The changes in FFA of smoked tuna in oil are given in Figures 35-36. The initial values were 1.68, 1.94, 1.23 and 2.17 mg % for the INOP, INST, IMST and INTL pouches which increased to 5.8, 6.32. 6.4 mg% after 12 months for INOP, INST and IMST pouches at ambient storage and 6.2, 7.12 and 7.13 mg % at accelerated temperature storage. Final values in INTL pouches after storage for ambient and accelerated temperature were 4.2 and 7.9 mg % Aubourg *et al.,* (1997) found that tuna processed in oil at an elevated temperature (130°C) developed a higher level of FFA compared to the lower temperature processes (110 °C and 121°C). Increased level of FFA formation was observed by Tanaka *et al.,* (1985) at lower temperatures of processing, due to the longer process time at these temperatures. In the present study also an increasing trend is seen in the values of FFA in all the pouches. Medina *et al.,* (1995) found that the extent and mechanism of lipid hydrolysis does not depend on the filling medium employed.

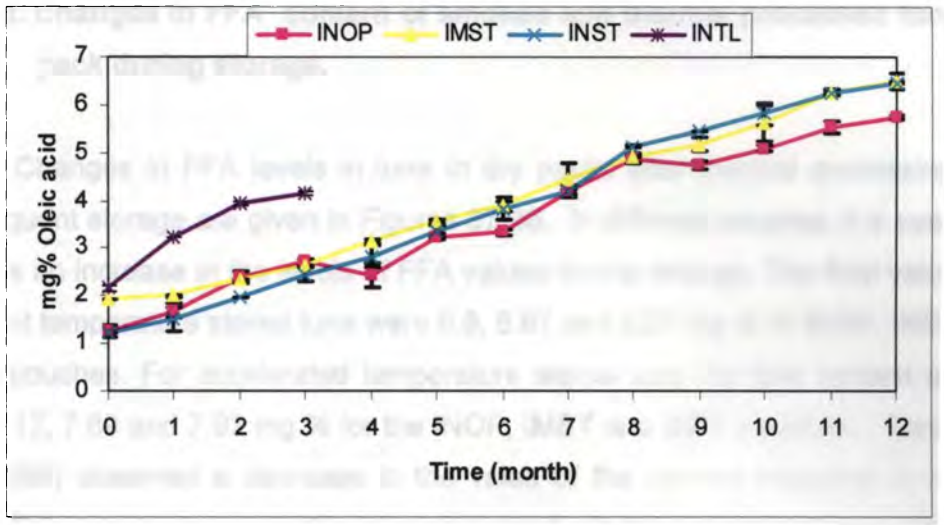


Fig. 35. Changes in FFA values of smoked and thermal processed tuna in oil in different pouches during storage at 28 ± 2°C.

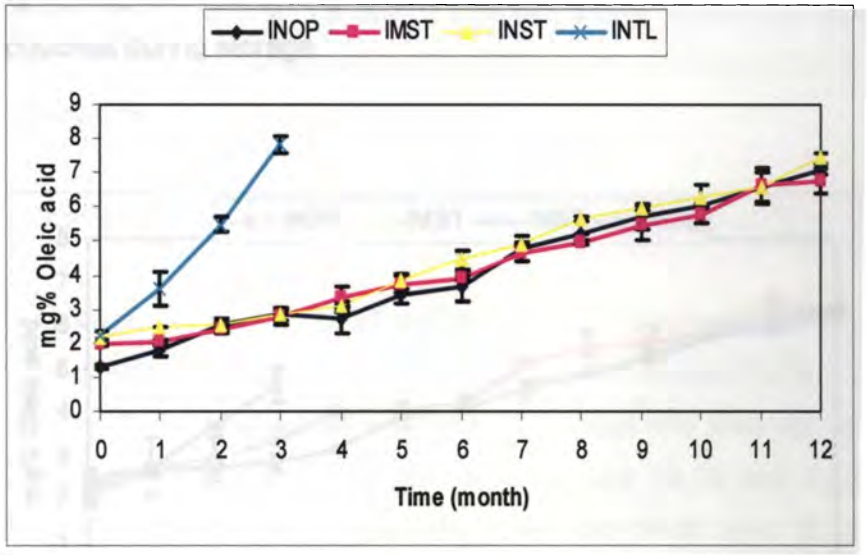


Fig. 36. Changes in FFA values of smoked and thermal processed tuna in oil in different pouches during storage at 37 ± 2°C.

4.6.3.3. Changes in FFA content of smoked and thermal processed tuna dry pack during storage.

Changes in FFA levels in tuna in dry packs after thermal processing and subsequent storage are given in Figures 37-38. In different pouches it is seen that there is an increase in the levels of FFA values during storage. The final values for ambient temperature stored tuna were 5.8, 5.87 and 6.21 mg % in INOP, INST and IMST pouches. For accelerated temperature stored tuna the final content of FFA was 6.12, 7.89 and 7.92 mg % for the INOP, IMST and INST pouches. Tanaka *et al.*, (1985) observed a decrease in the value of the canned mackerel in natural pack. This may be because the canning procedures for natural pack vary since no precooking is done. This canning process greatly increases the level of FFA in the meat (Medina *et al.*, 1995). In the present study the fish had already undergone precooking by way of hot smoking. Hence there is an increase of the FFA values in all the pouches during storage.

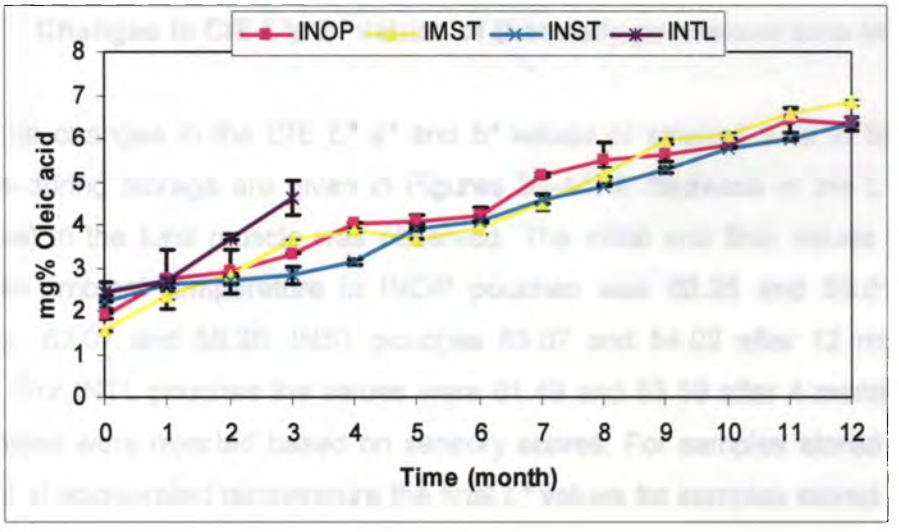


Fig. 37. Changes in FFA values of smoked and thermal processed tuna in dry pack in different pouches during storage at 28 ± 2°C

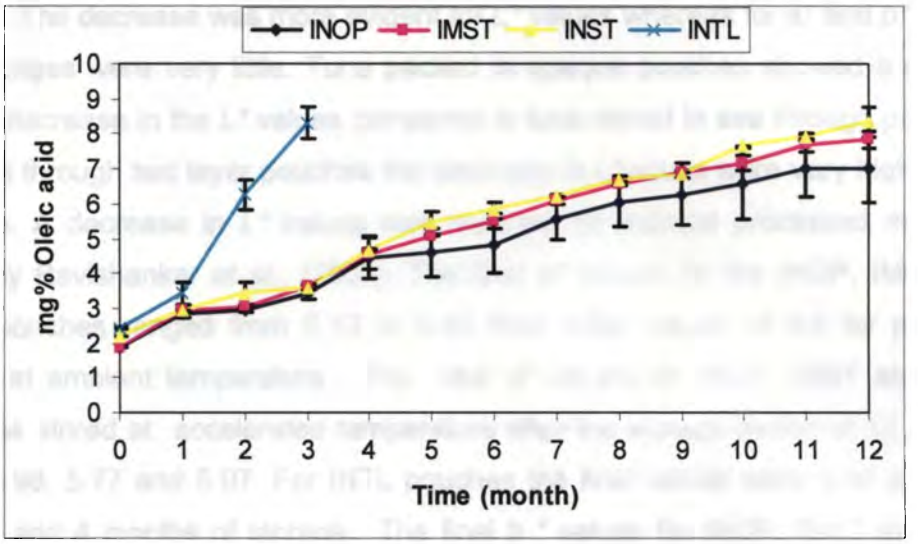


Fig. 38. Changes in FFA values of smoked and thermal processed tuna in dry pack in different pouches during storage at 37 ± 2°C.

4.6.4. Changes in CIE *L*a*b** values of smoked and thermal processed tuna during storage at ambient (28 ± 2°C) and accelerated (37 ± 2°C) temperature.

4.6.4.1. Changes in CIE *L*a*b** values of thermally processed tuna in brine

The changes in the CIE *L** *a** and *b** values of smoked tuna in brine and changes during storage are given in Figures 39-44. A decrease in the *L** values (lightness) in the tuna muscle was observed. The initial and final values for tuna stored at ambient temperature in INOP pouches was 63.25 and 58.01, IMST pouches 63.07 and 58.26, INST pouches 63.07 and 54.02 after 12 months of storage. For INTL pouches the values were 61.49 and 53.59 after 4 months when the samples were rejected based on sensory scores. For samples stored in brine and kept at accelerated temperature the final *L** values for samples stored in INOP pouches was 55.91, IMST pouches 58.26 and INST pouches was 54.02. The samples stored in INTL pouches had a *L** value of 53.21 after 2 months of storage after which the samples were rejected. Similarly there is a marginal decrease in the *a** values and a slight increase in the *b** values during the storage

period. The decrease was more evident for *L** values whereas for a* and *b** values the changes were very little. Tuna packed in opaque pouches showed a gradual slower decrease in the *L** values compared to tuna stored in see through pouches. For see through two layer pouches the decrease in *L**values were very high during storage. A decrease in *L** values was reported for thermal processed mackerel curry by Ravishankar *et al.*, (2008). The final a* values for the INOP, IMST and INST pouches ranged from 6.12 to 6.45 from initial values of 8.9 for pouches stored at ambient temperature . The final *a** values for INOP, IMST and INST pouches stored at accelerated temperature after the storage period of 12 months were 5.98, 5.77 and 5.07. For INTL pouches the final values were 5.46 and 6.25 after 2 and 4 months of storage. The final *b* * values for INOP, IMST and INST pouches stored at ambient temperature were 17.62, 18.95 and 18.01 and accelerated temperature stored samples were 19.23, 18.23 and 19.38. For INTL pouches the values were 17.94 and 17.89 after storage for 4 and 2 months respectively.

The changes in colour values during storage are mainly due to the exposure of the see through pouches to light and higher oxygen transmission rate of the pouches which enhances lipid oxidation. The browning substances formed due the various chemical reactions during thermal processing and storage period was found to have leached out into the filling medium giving a light brown colour to the brine solution. Similar changes in *L** and *a** values have been reported by Bindu *et al.*, (2007) in thermal processed clam meat during storage.

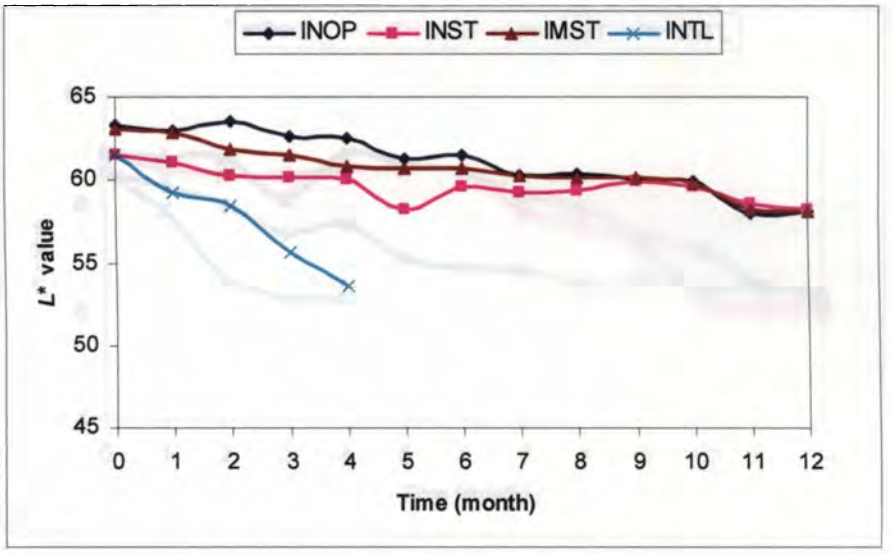


Fig. 39. Changes in *L values of smoked and thermal processed tuna in brine in different pouches during storage at 28±2°C**

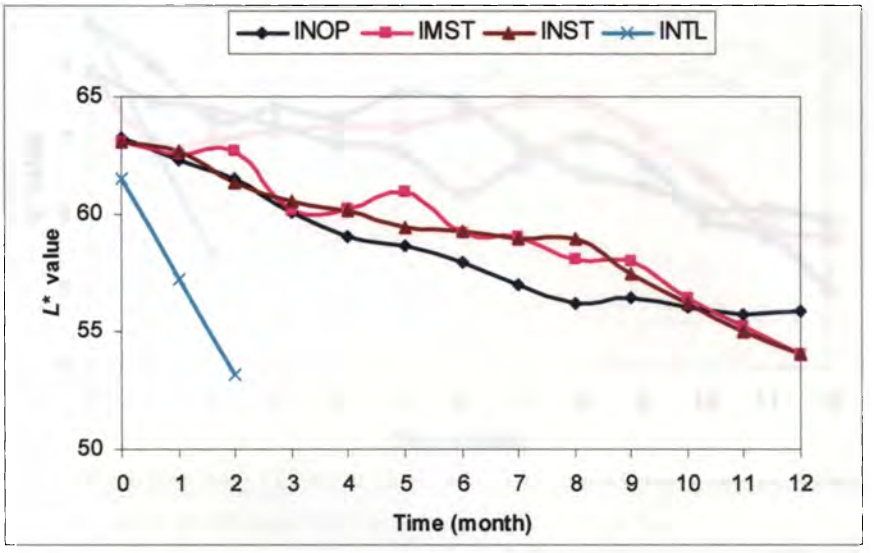


Fig. 40. Changes in *L values of smoked and thermal processed tuna in brine in different pouches during storage at 37± 2°C.**

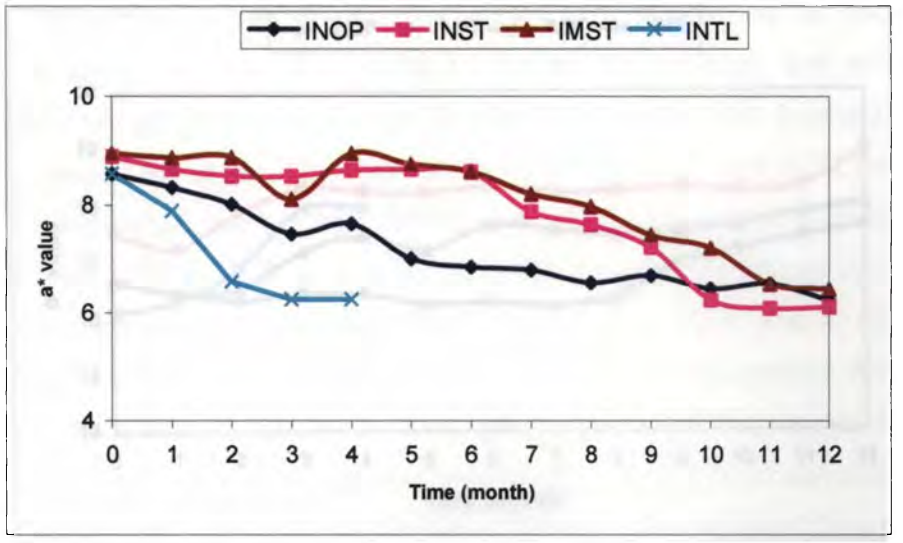


Fig. 41. Changes in a* values of smoked and thermal processed tuna in brine in different pouches during storage at 28±2°C

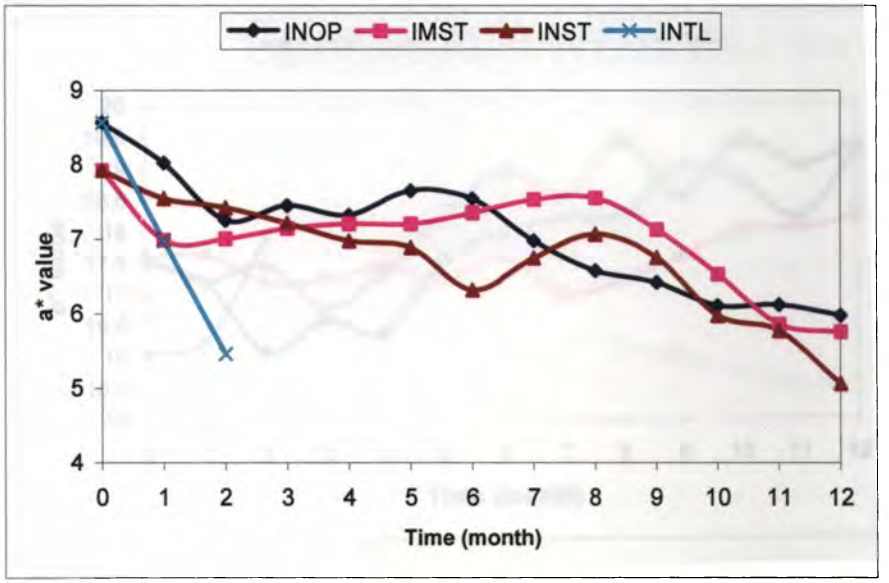


Fig. 42. Changes in *a values of smoked and thermal processed tuna in brine in different pouches during storage at 37± 2°C.**

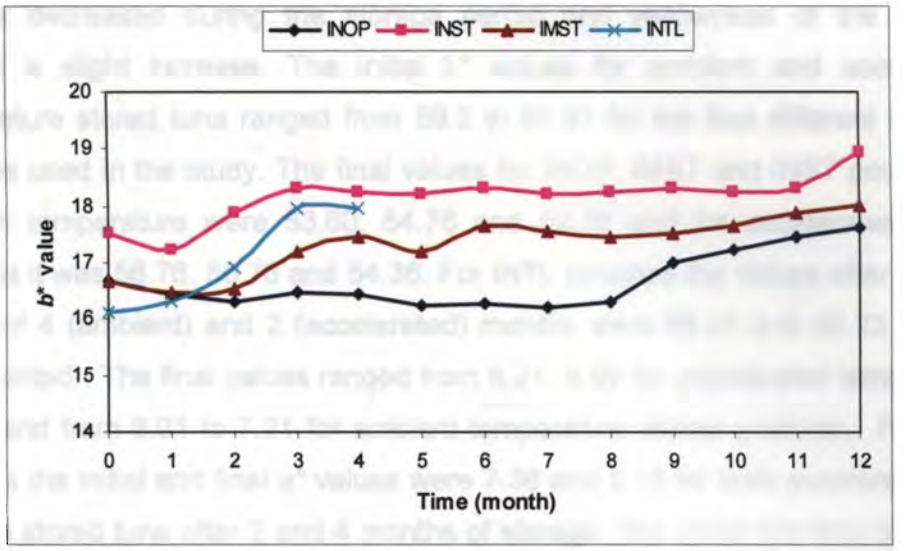


Fig. 43. Changes in b* values of smoked and thermal processed tuna in brine in different pouches during storage at 28±2°C

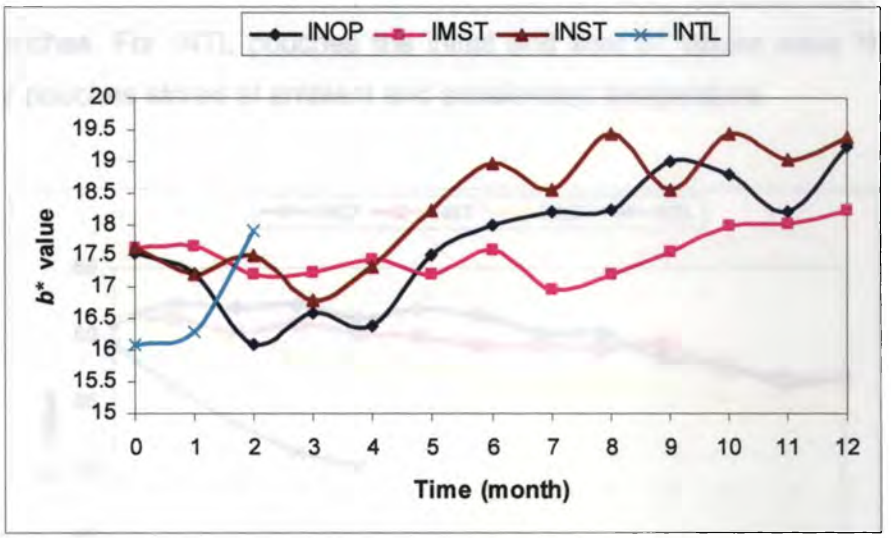


Fig. 44. Changes in *b values of smoked and thermal processed tuna in brine in different pouches during storage at 37± 2°C.**

4.6.4.2. Changes in CIE *L*a*b** values of thermally processed tuna in oil

The changes in the CIE *L*a*b** values of thermally processed tuna in oil are given in Figures 45-50 respectively. The *L** (lightness) value of the product and

redness decreased during the storage period and yellowness of the product showed a slight increase. The initial L* values for ambient and accelerated temperature stored tuna ranged from 59.2 to 61.61 for the four different types of pouches used in the study. The final values for INOP, IMST and INST pouches at ambient temperature were 53.60, 54.76 and 52.38 and for accelerated stored products it was 56.76, 56.76 and 54.38. For INTL pouches the values after storage period of 4 (ambient) and 2 (accelerated) months were 52.21 and 50.23 when it was rejected. The final values ranged from 5.21- 5.92 for accelerated temperature stored and from 6.01 to 7.21 for ambient temperature stored products. For INTL pouches the initial and final *a** values were 7.38 and 5.12 for both accelerated and ambient stored tuna after 2 and 4 months of storage. The initial and final *b** values for the pouches stored at ambient temperature ranged from 15.89 to 16.48 and 17.95 to 16.88 respectively. The final values for pouches stored at accelerated temperature were 17.98 for INOP pouches, 16.88 for IMST pouches and 17.95 for INST pouches. For INTL pouches the initial and final *b** values were 16.86 and 18.93 for pouches stored at ambient and accelerated temperature.

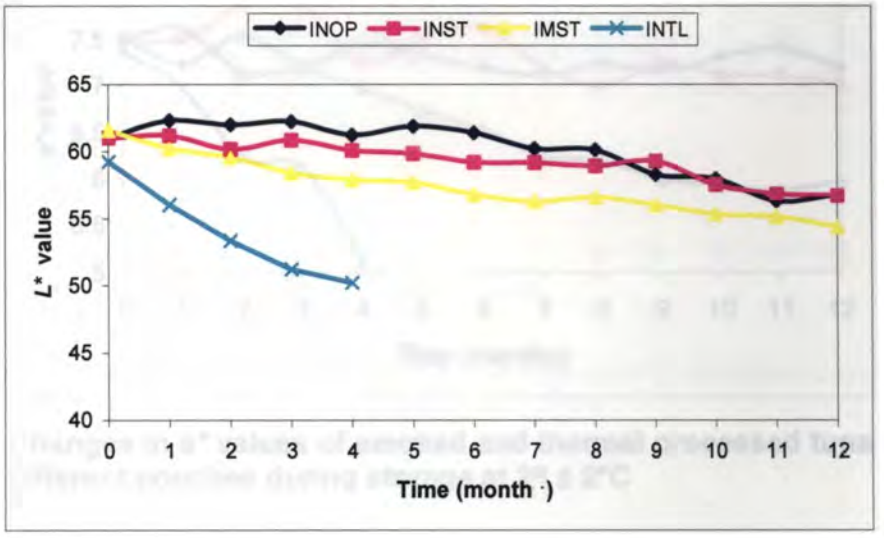


Fig. 45. Changes in *L values of smoked and thermal processed tuna in oil in different pouches during storage at 28 ± 2°C**

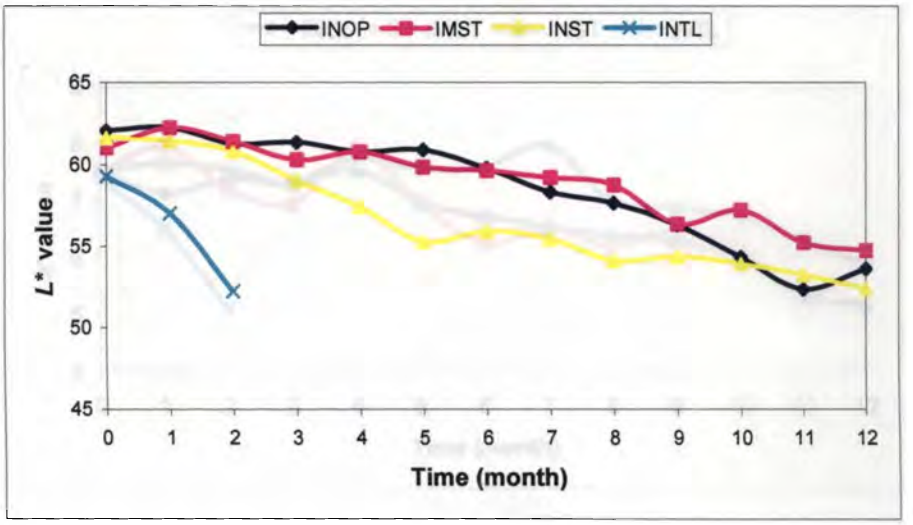


Fig. 46. Changes in *L values of smoked and thermal processed tuna in oil in different pouches during storage at 37 ± 2°C.**

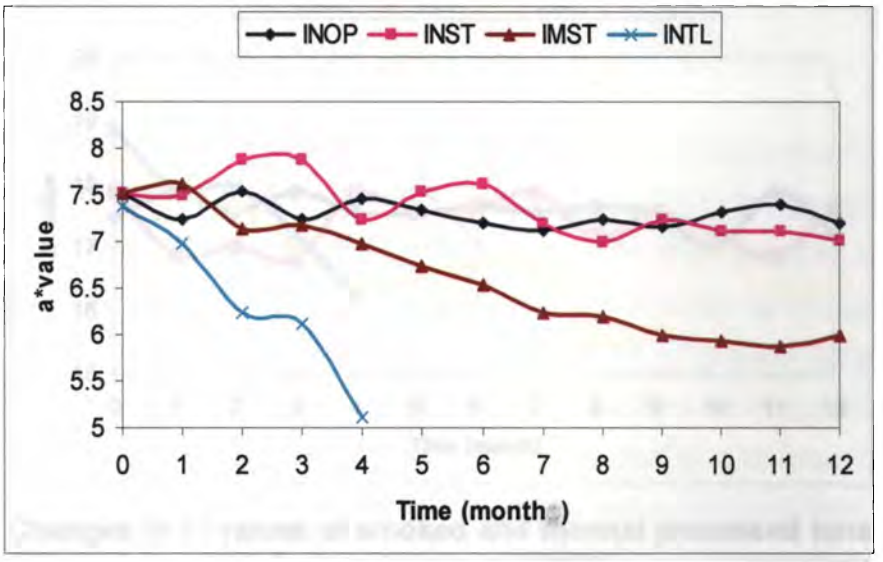


Fig. 47. Changes in a* values of smoked and thermal processed tuna in oil in different pouches during storage at 28 ± 2°C

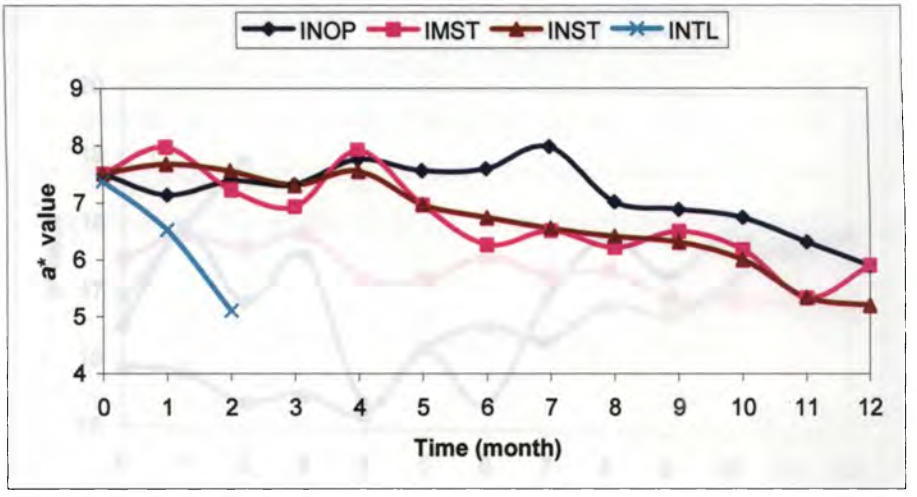


Fig. 48. Changes in *a values of smoked and thermal processed tuna in oil in different pouches during storage at 37± 2°C.**

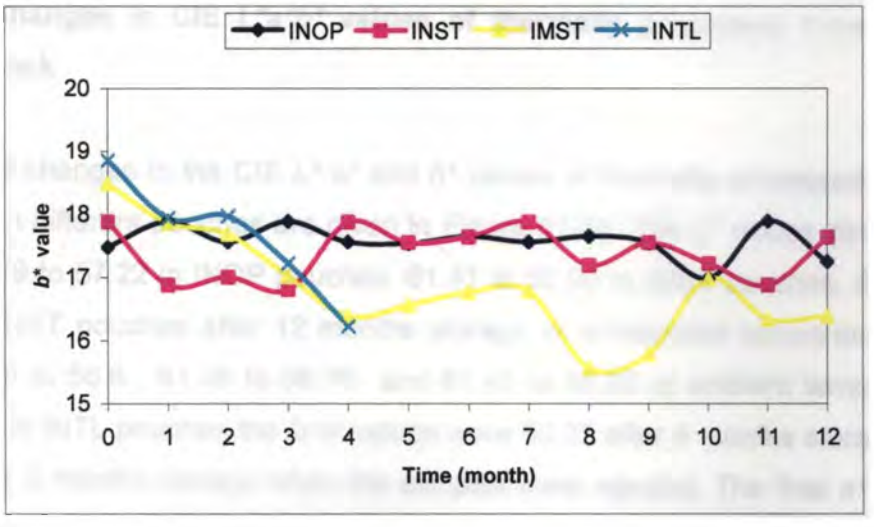


Fig. 49. Changes in *b values of smoked and thermal processed tuna in oil in different pouches during storage at 28±2°C**

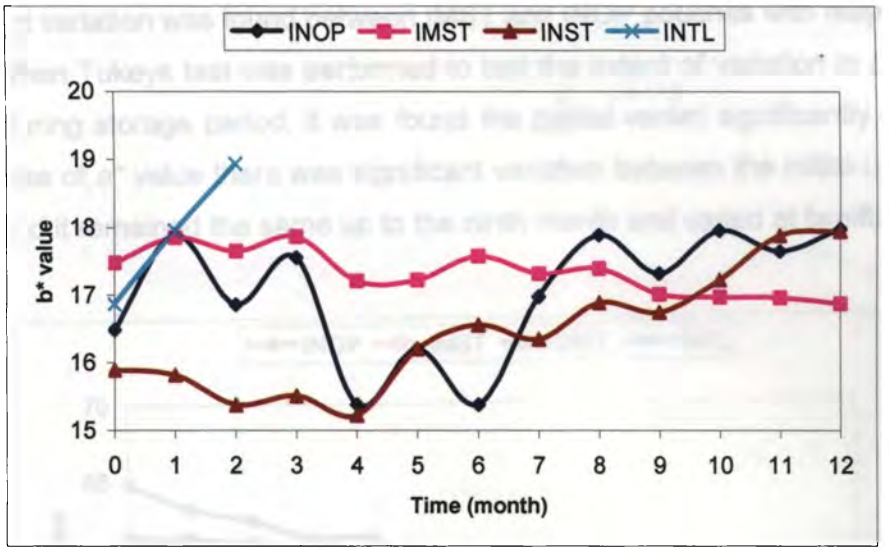


Fig. 50. Changes in *b values of smoked and thermal processed tuna in oil in different pouches during storage at 37± 2°C.**

4.6.4.2. Changes in CIE *L*a*b** values of thermally processed tuna in dry pack

The changes in the CIE *L** *a** and *b** values of thermally processed tuna in dry pack in different pouches are given in Figure 51-56. The *L** values decreased from 64.79 to 57.22 in INOP pouches, 61.41 to 52.90 in IMST pouches, 59.35 to 50.95 in INST pouches after 12 months storage at accelerated temperature and from 64.79 to 56.6 , 61.05 to 56.76 and 61.41 to 55.95 at ambient temperature storage. In INTL pouches the final values were 50.23 after 4 months storage and 52.21 after 2 months storage when the samples were rejected. The final *a** values for INOP, IMST and INST pouches stored at ambient and accelerated temperature ranged from 6.09 to 7.21 and *b** values were 18.56 to 20.89. The decrease in *a** (redness) and increase in *b**(yellowness) values of the products during storage were marginal and hence the changes were not prominent. These results correlated well with the sensory colour assessment by the panelist

*L*a*b** values varied significantly between the pouches ($p \leq .01$) and between the medium. Tukey's test was performed and it was found that no

significant variation was found between IMST and INOP pouches with respect to *a** value. When Tukeys test was performed to test the extent of variation in L* and b* values during storage period, it was found the period varied significantly ($p \leq .01$). In the case of *a** value there was significant variation between the initial up to third month and it remained the same up to the ninth month and varied at twelfth month.

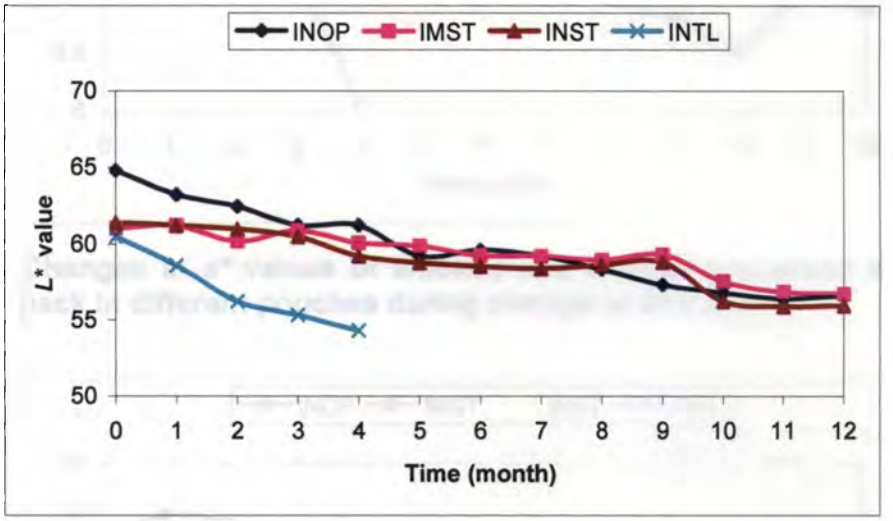


Fig. 51. Changes in *L values of smoked and thermal processed tuna dry pack in different pouches during storage at 28±2°C**

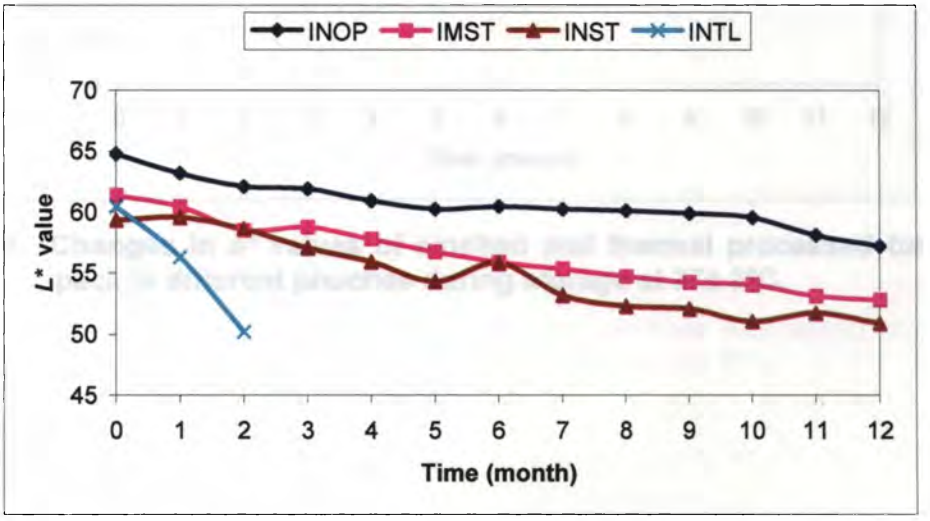


Fig. 52. Changes in *L values of smoked and thermal processed tuna dry pack in different pouches during storage at 37± 2°C.**

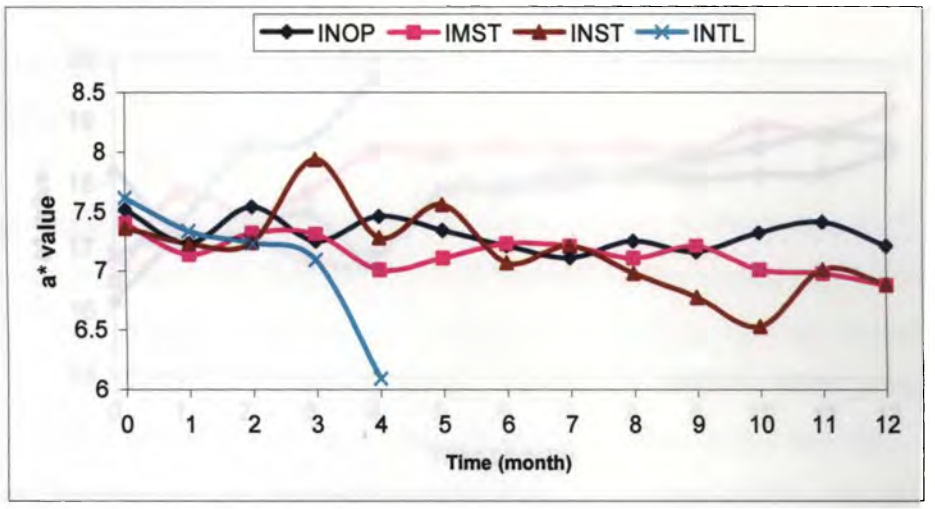


Fig. 53. Changes in *a values of smoked and thermal processed tuna dry pack in different pouches during storage at 28 ± 2°C**

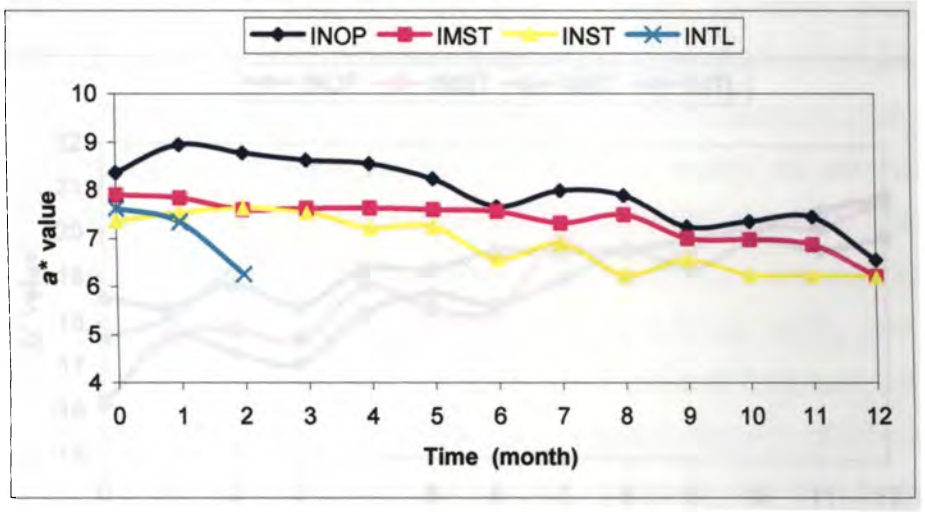


Fig. 54. Changes in *a values of smoked and thermal processed tuna dry pack in different pouches during storage at 37± 2°C.**

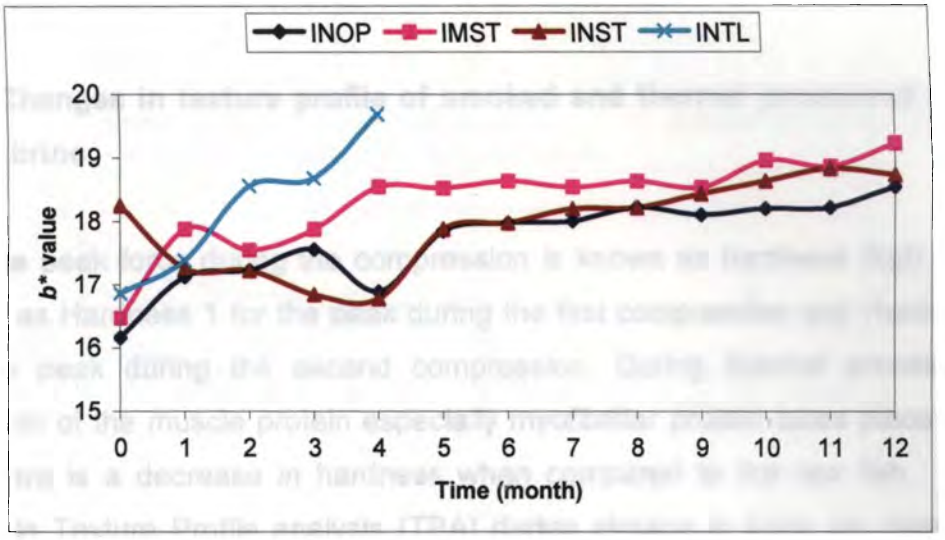


Fig. 55. Changes in b* value of smoked and thermal processed tuna dry pack in different pouches during storage at 28±2°C

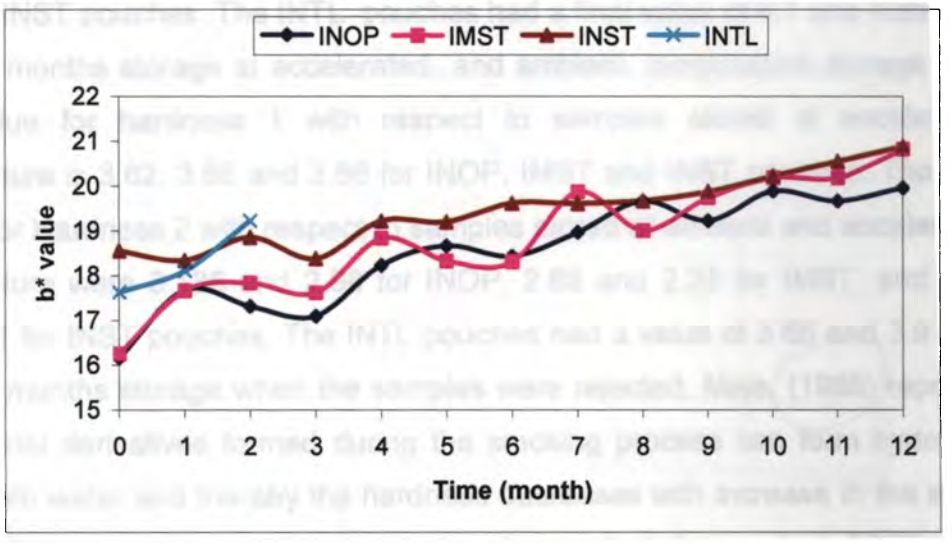


Fig. 56. Changes in *b values of smoked and thermal processed tuna dry pack in different pouches during storage at 37± 2°C.**

4.6.5. Changes in texture profile of smoked and thermal processed tuna during storage at ambient (28±2°C) and accelerated (37±2°C) temperature.

4.6.5.1. Changes in texture profile of smoked and thermal processed tuna in brine

The peak force during the compression is known as hardness (kgf). It is identified as Hardness 1 for the peak during the first compression and Hardness 2 for the peak during the second compression. During thermal processing dissociation of the muscle protein especially myofibrillar protein takes place and hence there is a decrease in hardness when compared to the raw fish. The changes in Texture Profile analysis (TPA) during storage in brine are given in Figures 57-64. The hardness I and hardness II decreased during storage. The initial and final values for hardness 1 during storage at ambient temperature is 5.17 and 3.82 for INOP pouches, 5.10 and 3.68 for IMST pouches , 5.03 and 3.56 for INST pouches. The INTL pouches had a final value of 4.1 and 4.55 after 2 and 4 months storage at accelerated and ambient temperature storage. The final value for hardness 1 with respect to samples stored at accelerated temperature is 3.62, 3.68 and 3.56 for INOP, IMST and INST pouches. The final values for Hardness 2 with respect to samples stored at ambient and accelerated temperature were 3.188 and 2.98 for INOP, 2.63 and 2.33 for IMST, and 2.67 and 2.51 for INST pouches. The INTL pouches had a value of 3.65 and 3.9 after 4 and 2 months storage when the samples were rejected. Maja, (1988) reported that phenol derivatives formed during the smoking process can form hydrogen bonds with water and thereby the hardness decreases with increase in the water content (Rongrong *et al.,* 1988) and during storage (Lakshman *et al.,* 2003). This can be one of the reasons for the decrease in the hardness during storage of thermal processed products in brine. Changes in other parameters like springiness and chewiness were found to decrease gradually during storage. Cohesiveness which is a measurement of the internal bonding remained more or less during the storage period. The springiness values for both accelerated and

ambient stored tuna varied from 2.01- 2.65 and the cohesiveness values from 0.21 to 0.31. The final values for chewiness ranged from 2.04 to 2.42 in all the pouches after 12 months of storage.

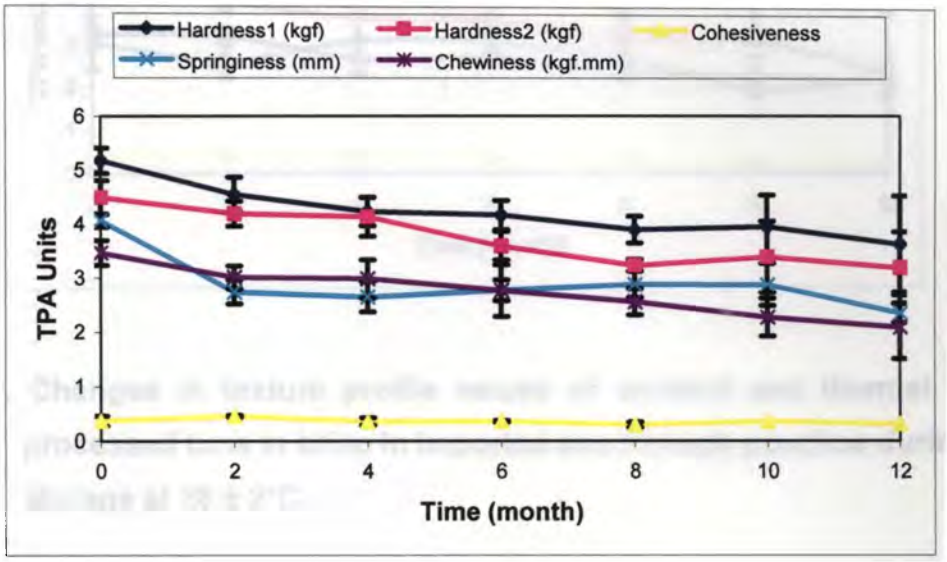


Fig. 57. Changes in texture profile values of smoked and thermal processed tuna in brine in indigenous opaque pouches during storage at 28 ± 2°C.

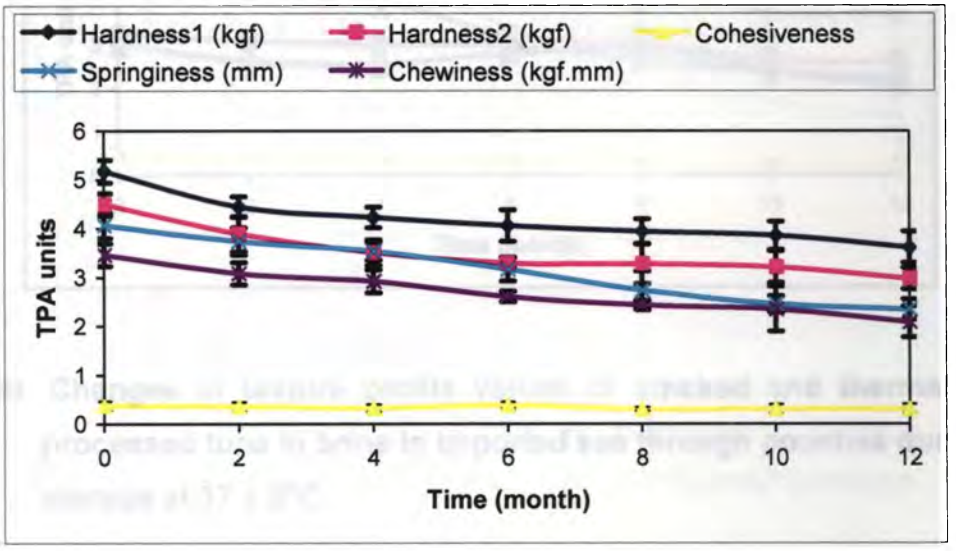


Fig. 58. Changes in texture profile values of smoked and thermal processed tuna in brine in indigenous opaque pouches during storage at 37 ± 2°C.

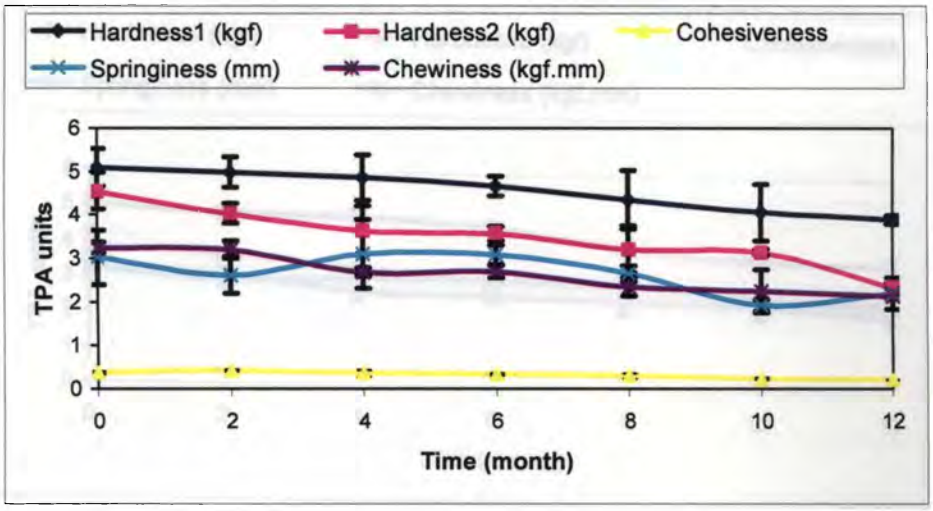


Fig. 59. Changes in texture profile values of smoked and thermal processed tuna in brine in imported see through pouches during storage at 28 ± 2°C.

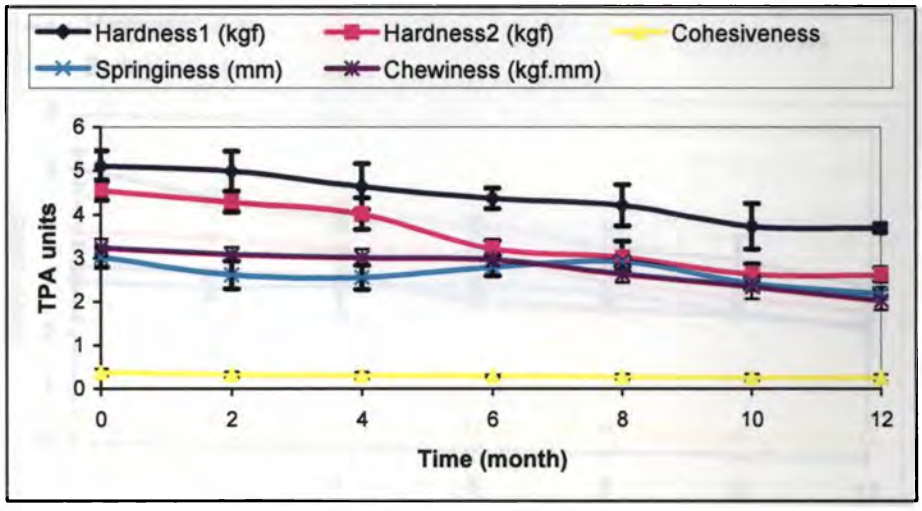


Fig. 60. Changes in texture profile values of smoked and thermal processed tuna in brine in imported see through pouches during storage at 37 ± 2°C.

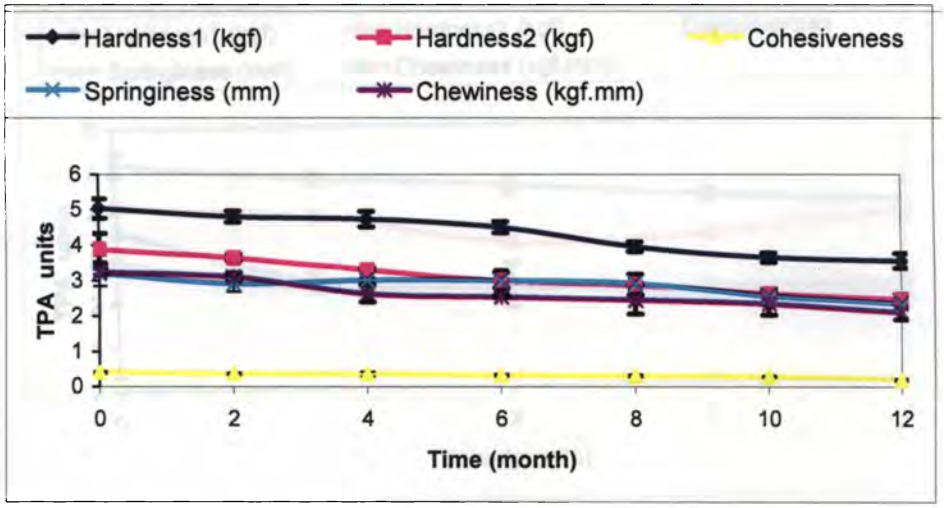


Fig. 61. Changes in texture profile values of smoked and thermal processed tuna in brine in indigenous see through pouches during storage at 28 ± 2°C.

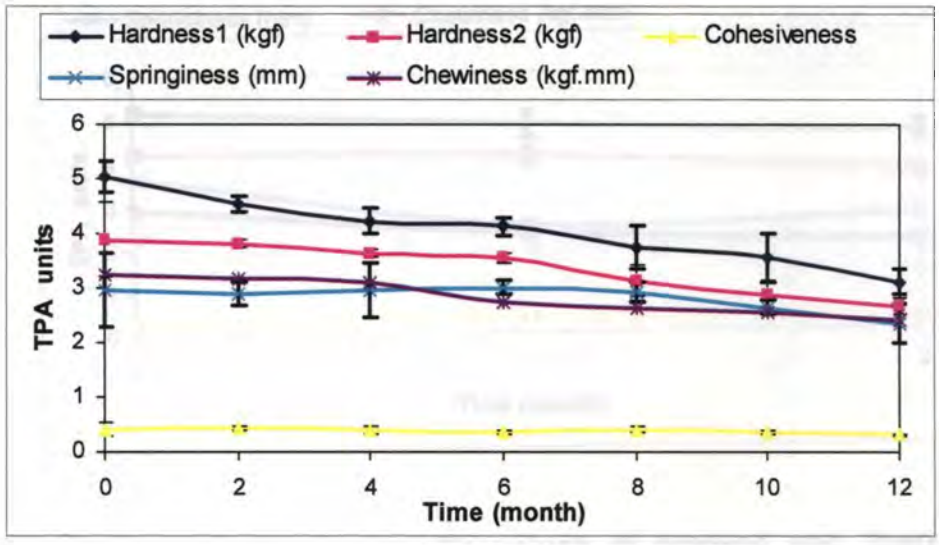


Fig. 62. Changes in texture profile values of smoked and thermal processed tuna in brine in indigenous see through pouches during storage at 37± 2°C.

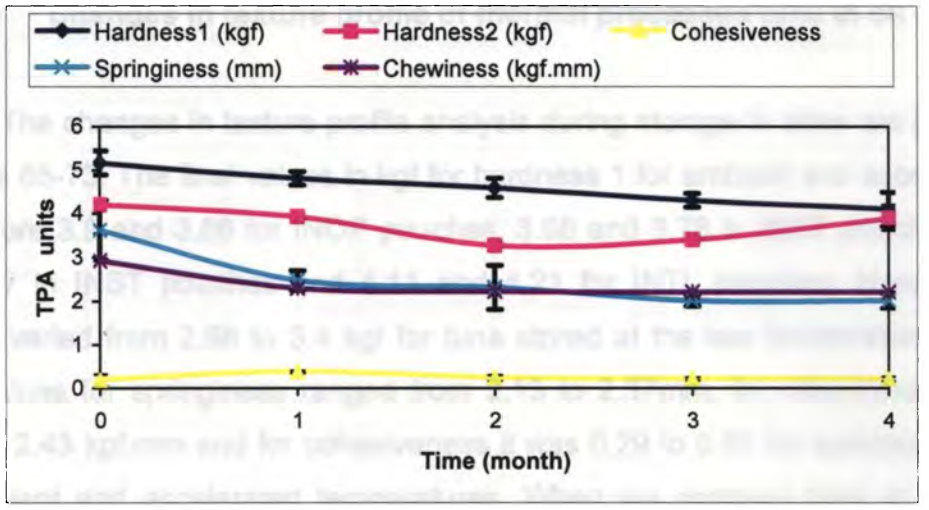


Fig. 63. Changes in texture profile values of smoked and thermal processed tuna in brine in indigenous two layer pouches during storage at 28 ± 2°C.

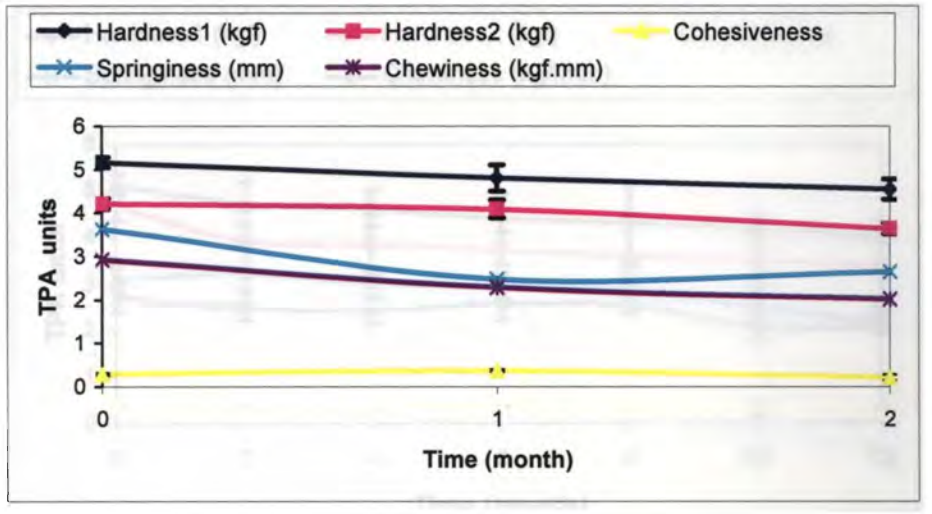


Fig. 64. Changes in texture profile values of smoked and thermal processed tuna in brine in indigenous two layer pouches during storage at 37 ± 2°C.

4.6.5.2. Changes in texture profile of thermal processed tuna in oil

The changes in texture profile analysis during storage in brine are given in Figures 65-73. The final values in kgf for hardness 1 for ambient and accelerated tuna were 3.9 and 3.66 for INOP pouches, 3.98 and 3.78 in IMST pouches, 3.9 and 3.7 in INST pouches and 4.11 and 4.21 for INTL pouches. Hardness 2 values varied from 2.98 to 3.4 kgf for tuna stored at the two temperatures. The final values for springiness ranged from 2.13 to 2.37mm, for chewiness it was 2.09 to 2.43 kgf.mm and for cohesiveness it was 0.29 to 0.31 for samples stored at ambient and accelerated temperatures. When we compare tuna in oil and brine it is seen that tuna processed in oil medium had a lower hardness value due to the longer processing time taken to reach the Fo value of 10. Similar results have seen reported by Xavier *et al.*, (2007).

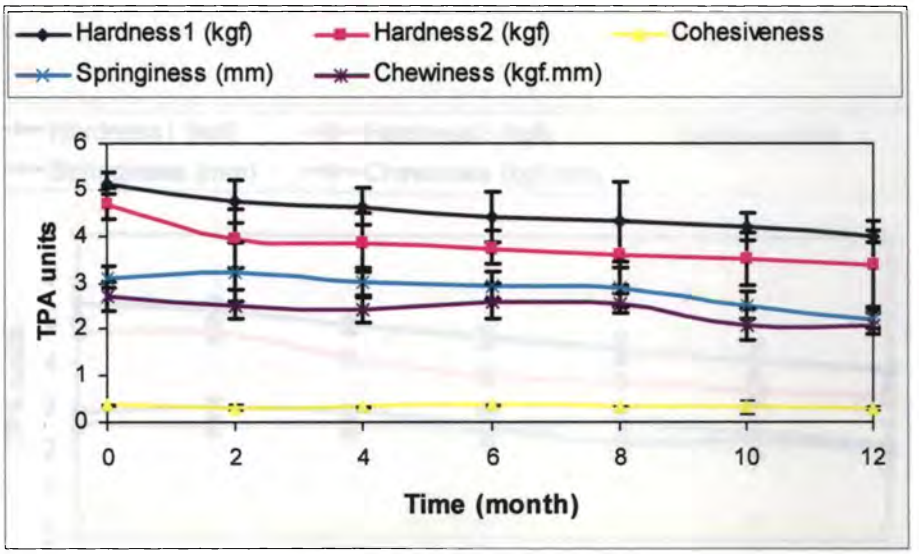


Fig. 65. Changes in texture profile values of smoked and thermal processed tuna in oil in indigenous opaque pouches during storage at 28 ± 2°C.

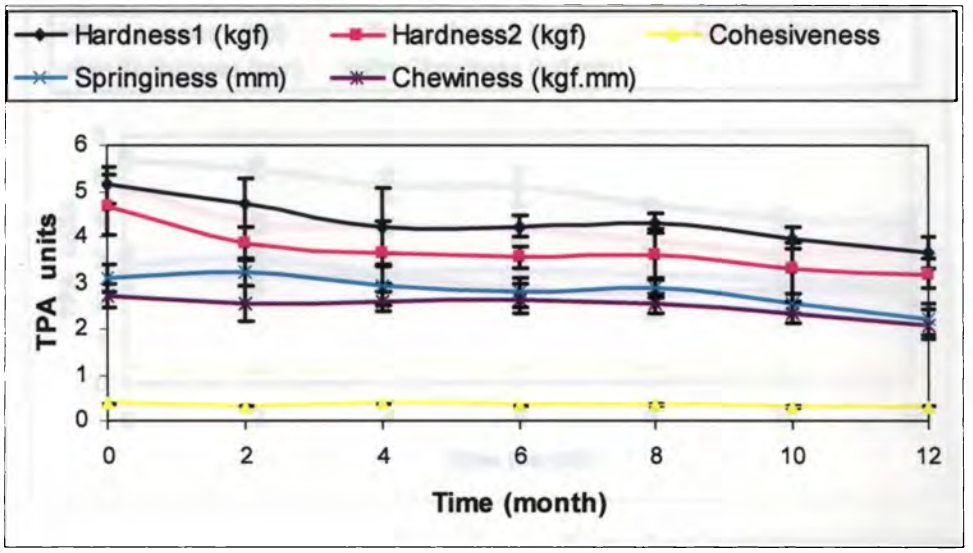


Fig. 66. Changes in texture profile values of smoked and thermal processed tuna in oil in indigenous opaque pouches during storage at 37± 2°C.

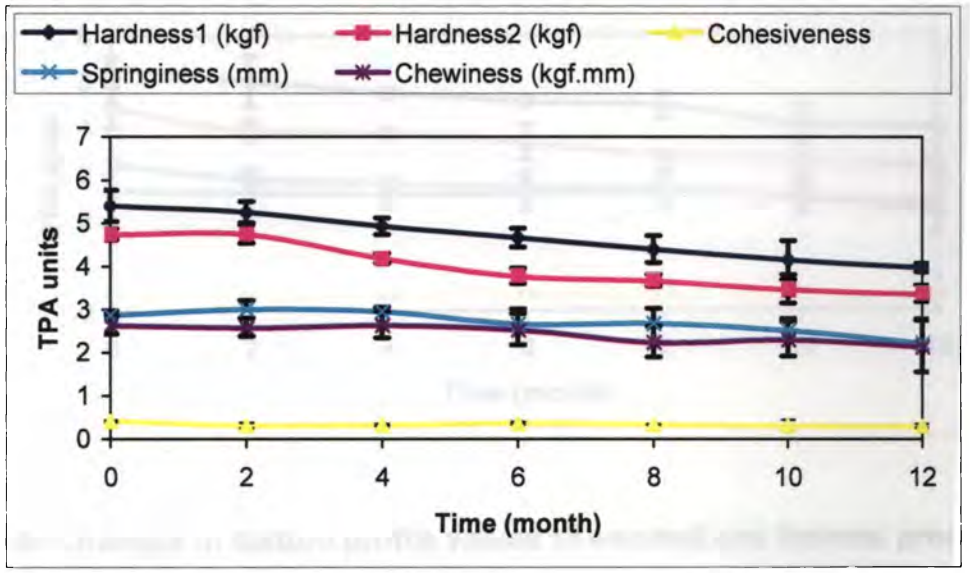


Fig. 67. Changes in texture profile values of smoked and thermal processed tuna in oil in imported see through pouches during storage at 28 ± 2°C.

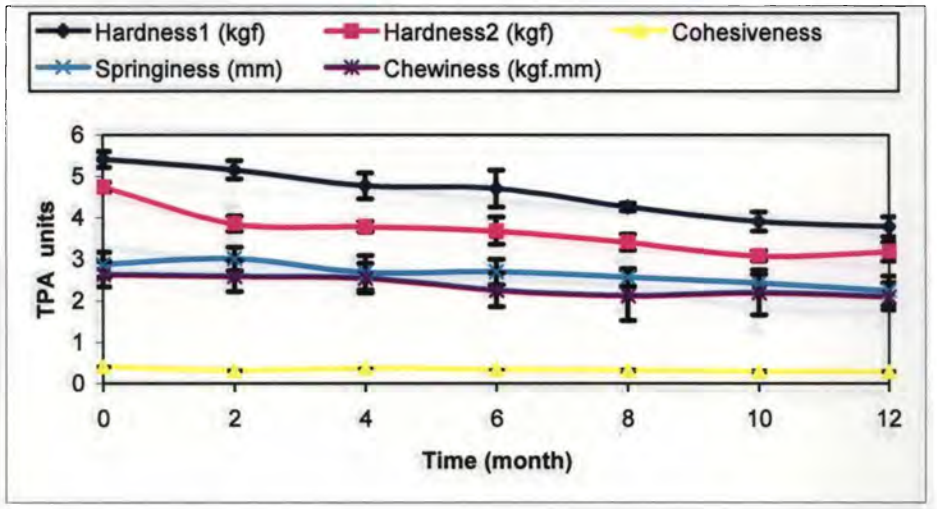


Fig. 68. Changes in texture profile values of smoked and thermal processed tuna in oil in imported see through pouches during storage at 37± 2°C

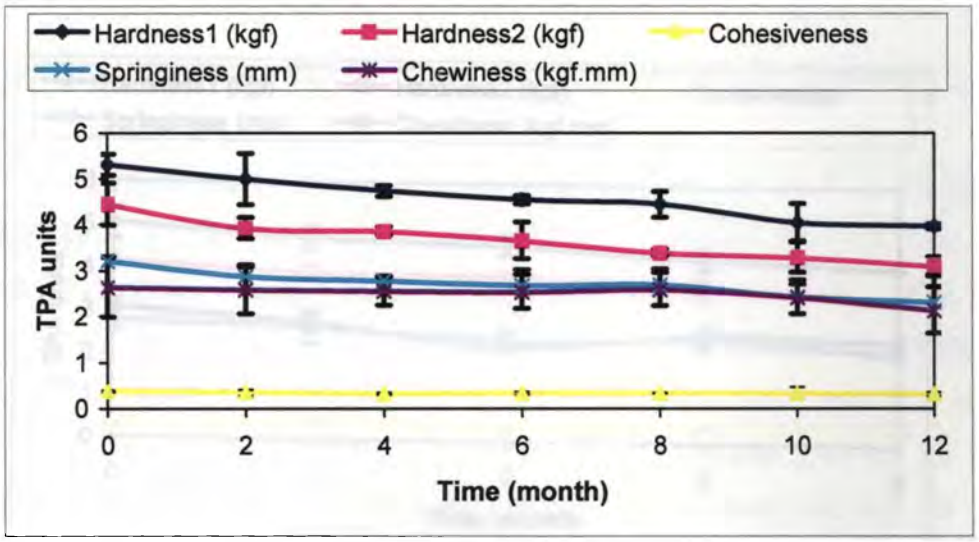


Fig. 69. Changes in texture profile values of smoked and thermal processed tuna in oil in indigenous see through pouches during storage at 28 ± 2°C.

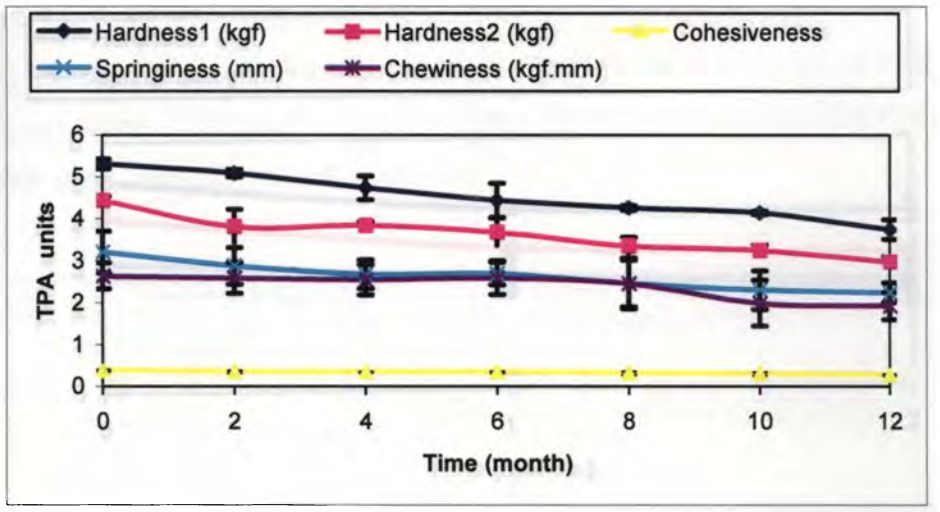


Fig. 70. Changes in texture profile values of smoked and thermal processed tuna in oil in indigenous see through pouches during storage at 37± 2°C.

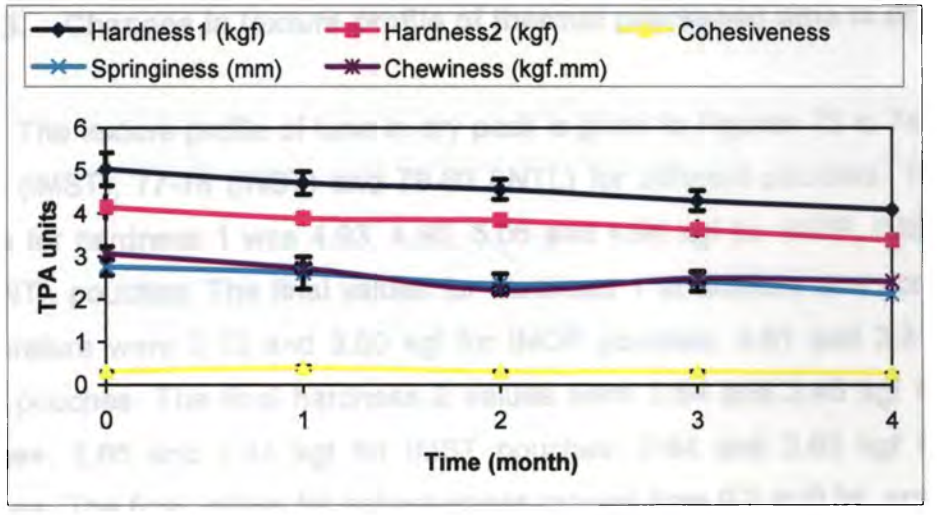


Fig. 71. Changes in texture profile values of smoked and thermal processed tuna in oil in indigenous two layer pouches during storage at 28 ± 2°C.

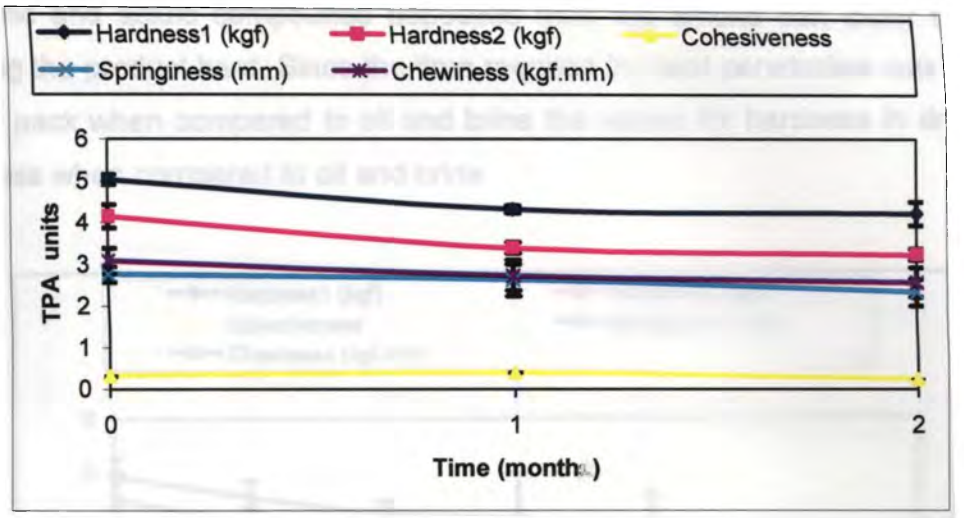


Fig. 72. Changes in texture profile values of smoked and thermal processed tuna in oil in indigenous two layer pouches during storage at 37 ± 2°C.

4.6.5.3. Changes in texture profile of thermal processed tuna in dry pack

The texture profile of tuna in dry pack is given in Figures 73 to 74 (INOP), 75-76 (IMST), 77-78 (INST) and 79-80 (INTL) for different pouches. The initial values for hardness 1 was 4.93, 4.90, 5.06 and 4.94 kgf for INOP, IMST, INST and INTL pouches. The final values for hardness 1 at ambient and accelerated temperature were 3.73 and 3.50 kgf for INOP pouches, 3.61 and 3.31 kgf for IMST pouches. The final hardness 2 values were 2.84 and 2.45 kgf for INOP pouches, 2.65 and 2.45 kgf for IMST pouches, 2.64 and 2.63 kgf for INST pouches. The final values for cohesiveness ranged from 0.2 to 0.34, springiness from 1.56 to 2.61mm and chewiness from 1.95 to 2.54kgf.mm. The final values in hardness I was found to be 3.24 and 3.54kgf when pouches were rejected after 2 and 4 months. Similarly hardness 2 values were 2.32 and 2.98 kgf, cohesiveness was 0.2, springiness 2.1 and 2.01 mm and chewiness 2.54 and 2.02 kgf.mm for samples stored at ambient and accelerated temperature. The presence of salt and deposits from smoking process may have affected the texture of the tuna products. Salt can affect the texture of the meat by tenderizing the muscle and

phenolic and acidic compounds deposited from the smoke can lower the pH making the product hard. Since the time required for heat penetration was longer in dry pack when compared to oil and brine the values for hardness in dry pack was less when compared to oil and brine.

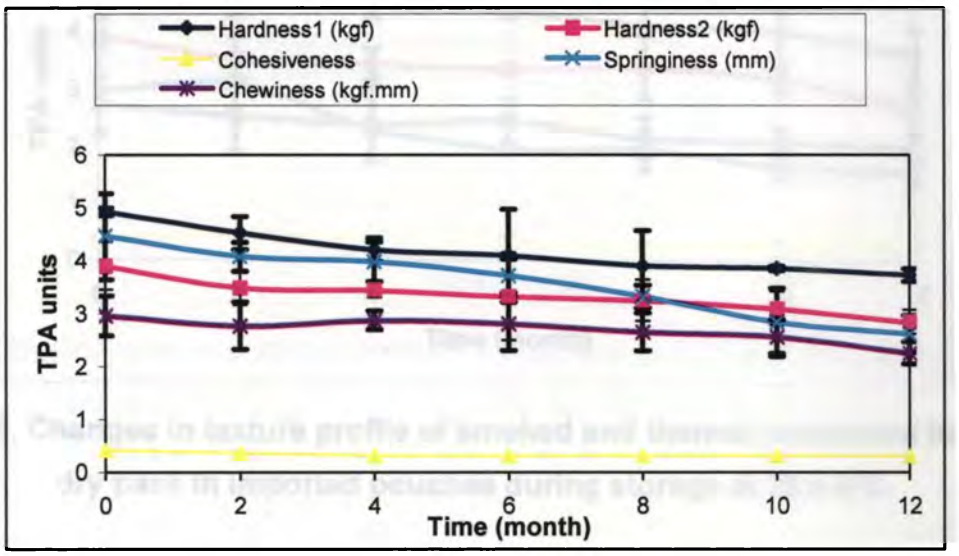


Fig.73. Changes in texture profile of smoked and thermal processed tuna in dry pack in indigenous opaque pouches during storage at 28 ± 2°C.

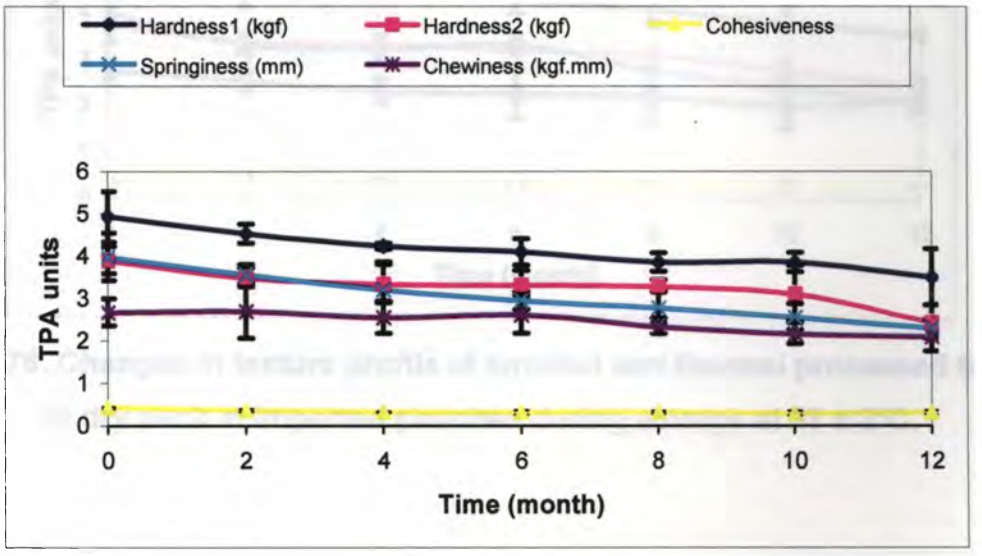


Fig.74. Changes in texture profile of smoked and thermal processed tuna in dry pack in indigenous opaque pouches during storage at 37± 2°C

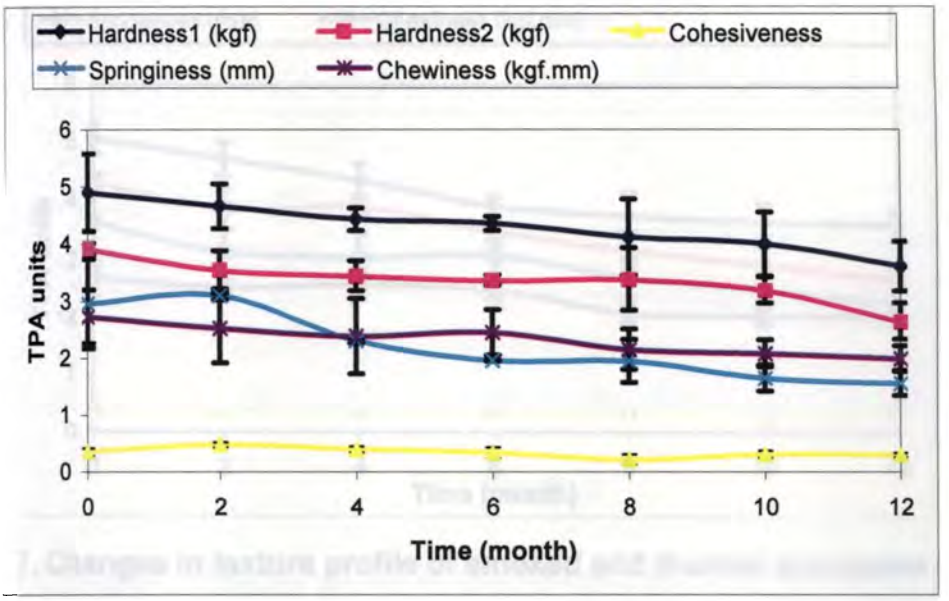


Fig.75. Changes in texture profile of smoked and thermal processed tuna in dry pack in imported pouches during storage at 28 ± 2°C.

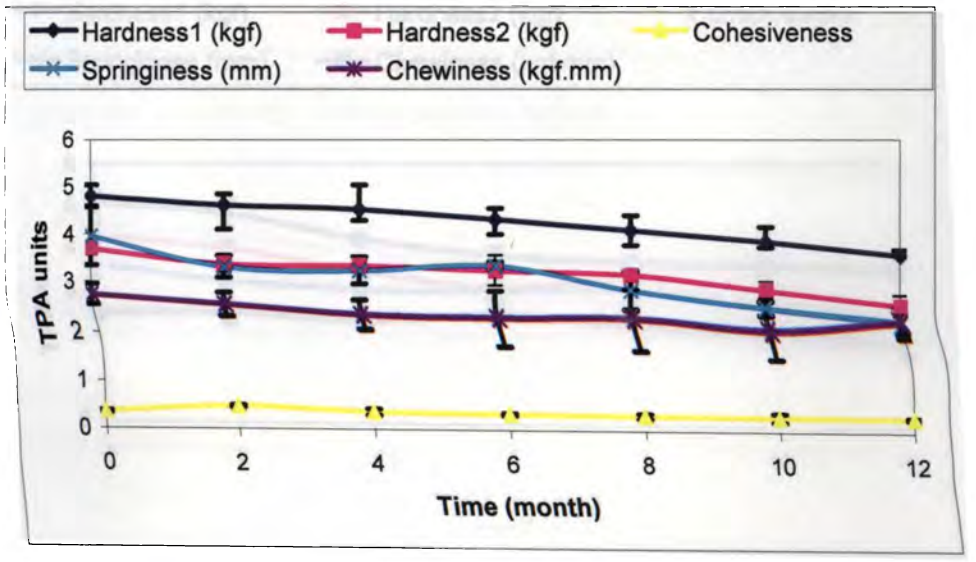


Fig. 76. Changes in texture profile of smoked and thermal processed tuna in dry pack in imported pouches during storage at 37 ± 2°C.

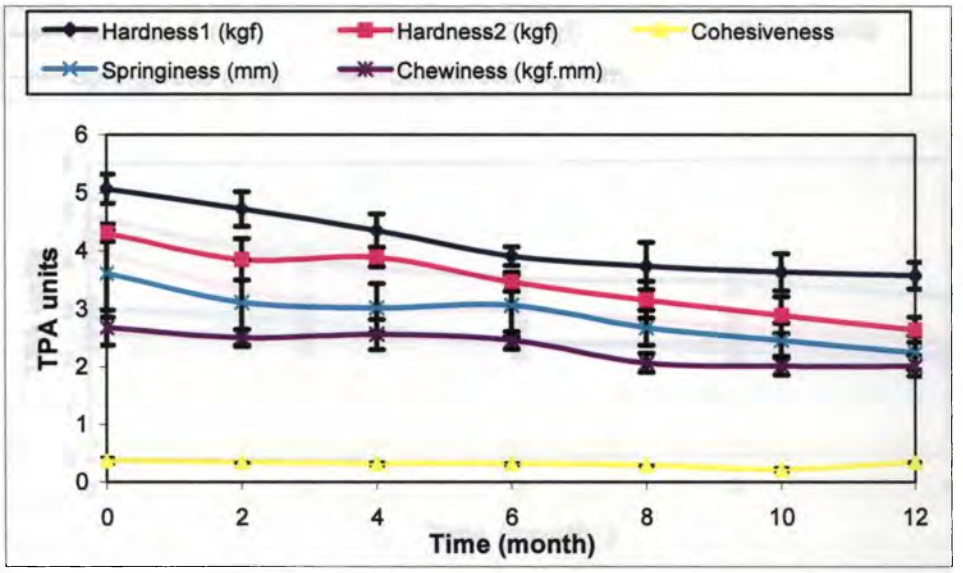


Fig. 77. Changes in texture profile of smoked and thermal processed tuna in dry pack in indigenous pouches during storage at 28 ± 2°C.

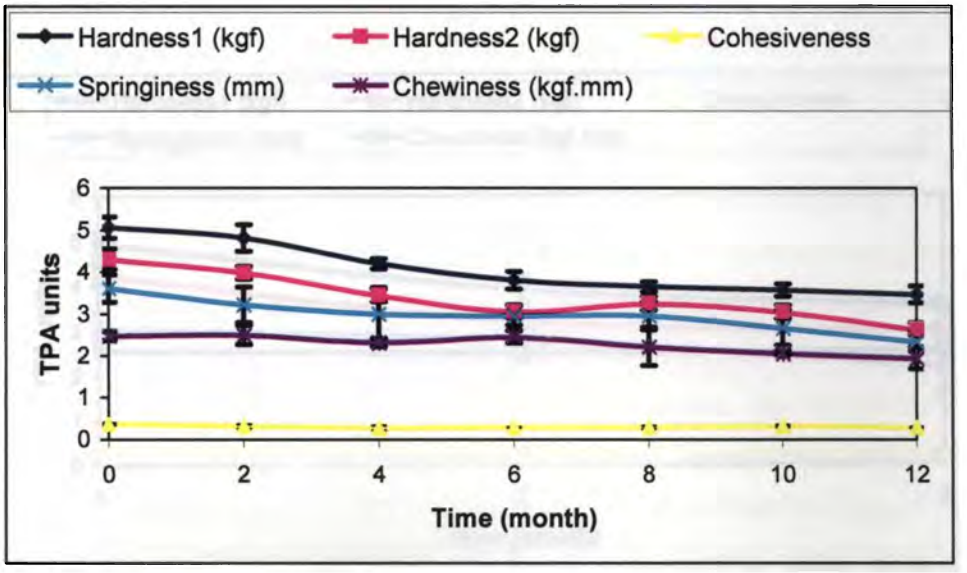


Fig. 78. Changes in texture profile of smoked and thermal processed tuna in dry pack in indigenous pouches during storage at 37 ± 2°C.

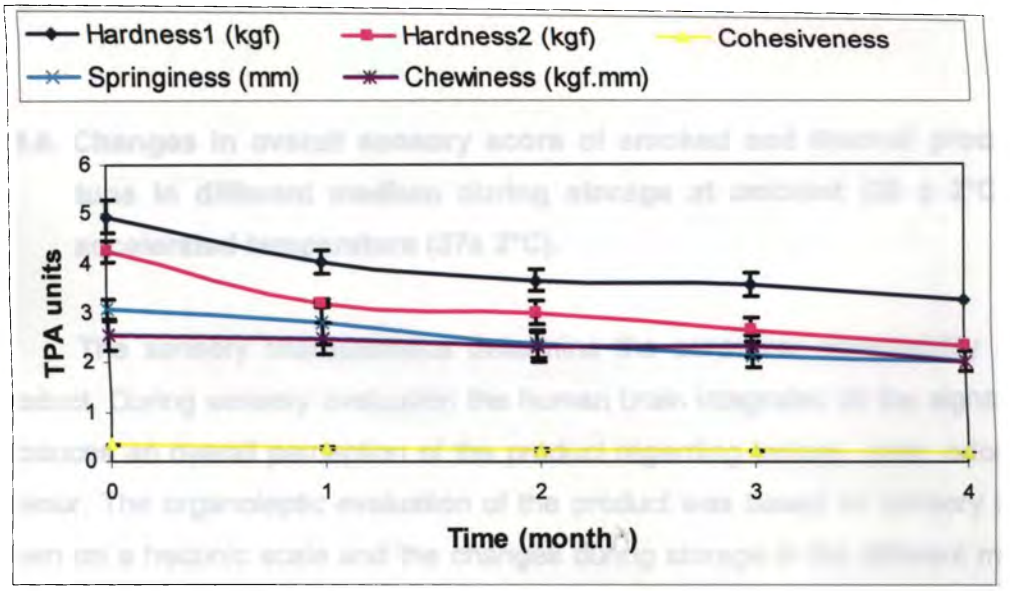


Fig. 79. Changes in texture profile of smoked and thermal processed tuna in dry pack in indigenous two layer pouches during storage at 28 ± 2°C.

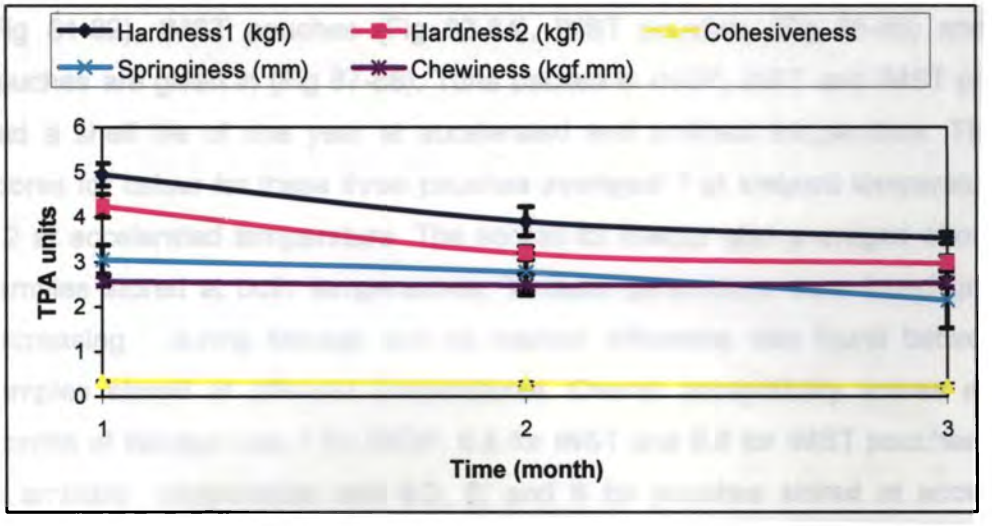


Fig. 80. Changes in texture profile of smoked and thermal processed tuna in dry pack in indigenous two layer pouches during storage at 37 ± 2°C.

4.6.6. Changes in overall sensory score of smoked and thermal processed tuna in different medium during storage at ambient (28 ± 2°C) and accelerated temperature (37± 2°C).

The sensory characteristics determine the consumer acceptability of the product. During sensory evaluation the human brain integrates all the signals and produces an overall perception of the product regarding texture, color, odour and flavour. The organoleptic evaluation of the product was based on sensory scores given on a hedonic scale and the changes during storage in the different medium were recorded. An overall acceptability score was derived based on different individual scores.

4.6.6.1. Changes in overall sensory score of smoked tuna packed in brine

Changes in overall sensory score of smoked tuna in brine for INOP pouches (Fig 81-82), IMST pouches (Fig 83-84), INST pouches (Fig 85-86) and INTL pouches are given in (Fig 87-88). Tuna packed in INOP, INST and IMST pouches had a shelf life of one year at accelerated and ambient temperature. The final scores for colour for these three pouches averaged 7 at ambient temperature and 6.2 at accelerated temperature. The scores for flavour also averaged about 7 for samples stored at both temperatures. Textural parameters were found gradually decreasing during storage and no marked difference was found between the samples stored at different temperatures. Overall acceptability scores after 12 months of storage was 7 for INOP, 6.8 for INST and 6.8 for IMST pouches stored at ambient temperature and 6.2, 6, and 6 for pouches stored at accelerated temperature. For INTL pouches scores at the point of rejection was 4 after storage for 2 months at accelerated and 4 months at ambient temperature. A gradual decreasing trend was noticed for all the parameters during the storage period. For flavour and colour the decreasing trend was more pronounced in INTL pouches due to higher permeability of the pouches used in the study.

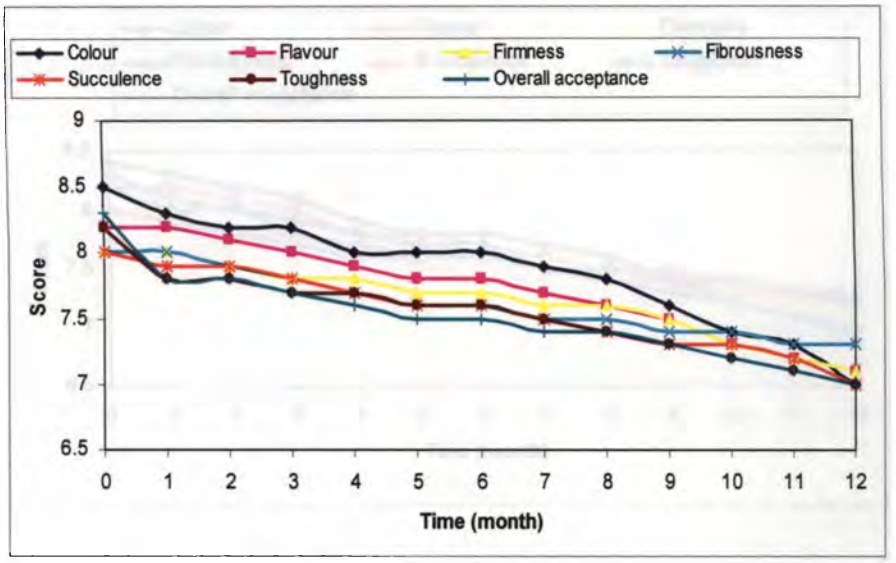


Fig. 81. Changes in sensory values of smoked and thermal processed tuna in brine in indigenous opaque pouches during storage at 28 ± 2°C.

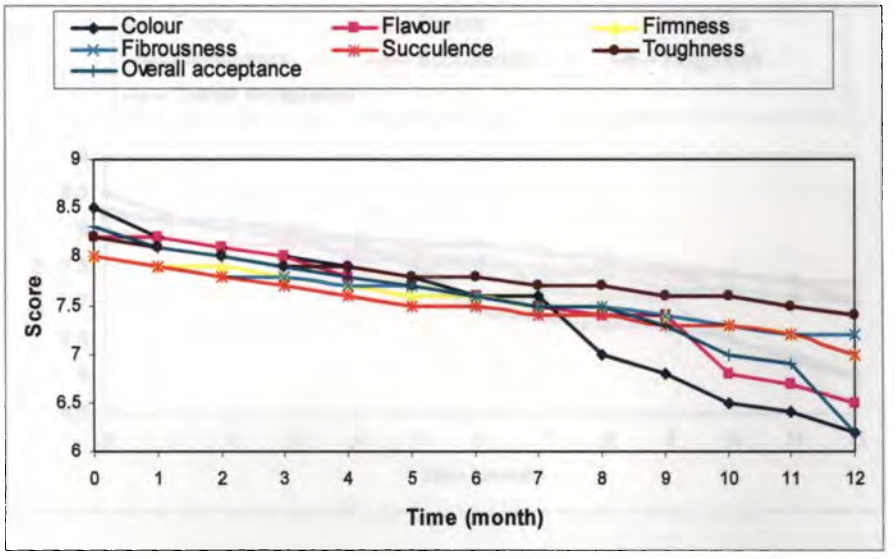


Fig. 82. Changes in sensory values of smoked and thermal processed tuna in brine in indigenous opaque pouches during storage at 37 ± 2°C

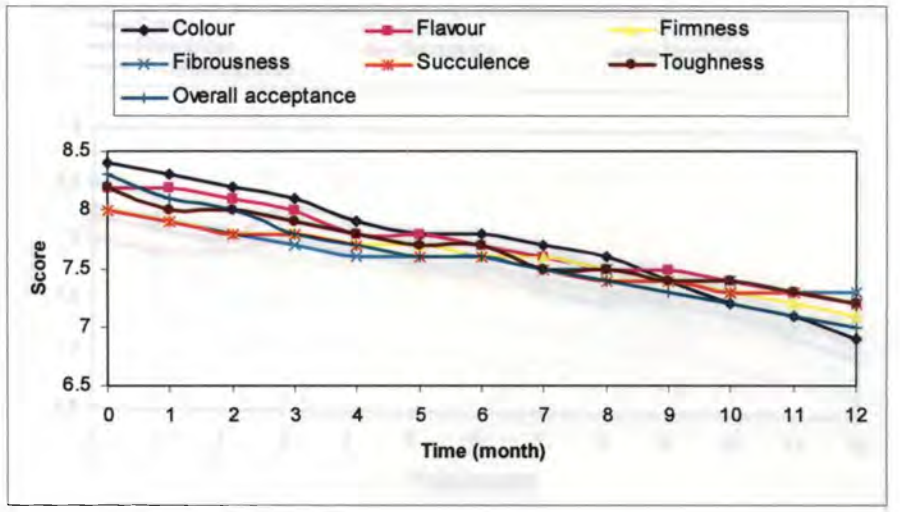


Fig. 83. Changes in sensory values of smoked and thermal processed tuna in brine in indigenous see through pouches during storage at 28 ± 2°C.

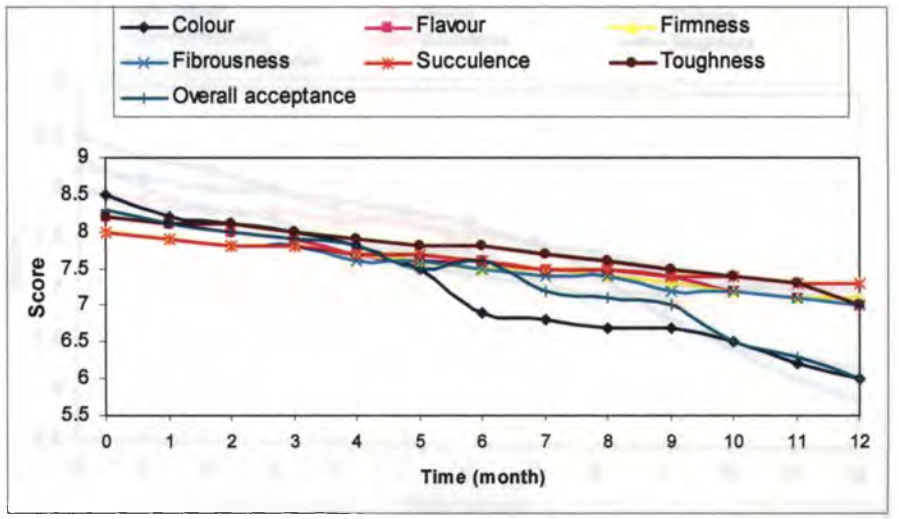


Fig. 84. Changes in sensory values of smoked and thermal processed tuna in brine in indigenous see through pouches during storage at 37 ± 2°C

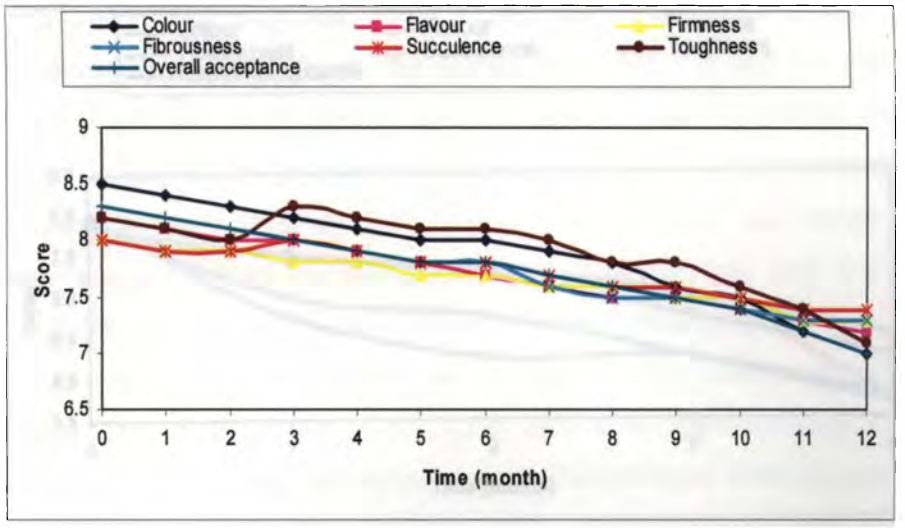


Fig. 85. Changes in sensory values of smoked and thermal processed tuna in brine in imported see through pouches during storage at 28 ± 2°C.

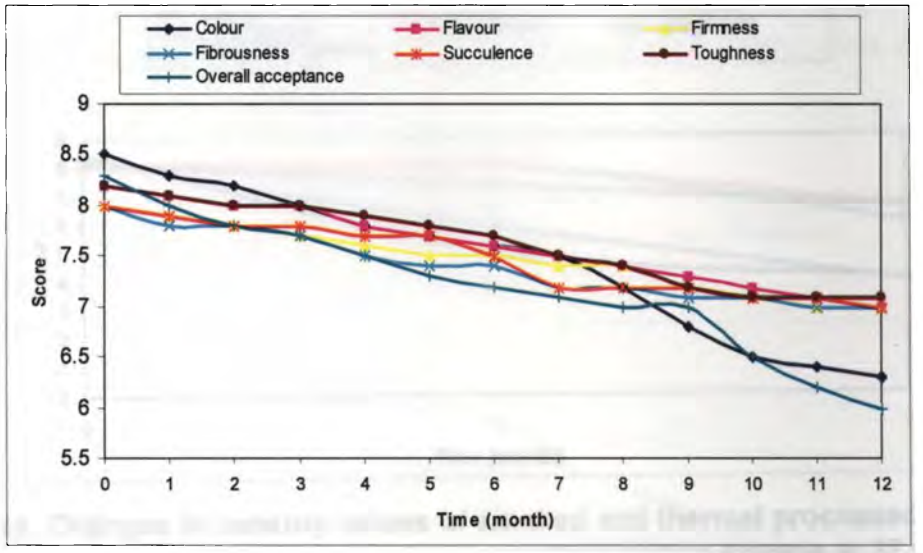


Fig. 86. Changes in sensory values of smoked and thermal processed tuna in brine in imported see through pouches during storage at 37 ± 2°C.

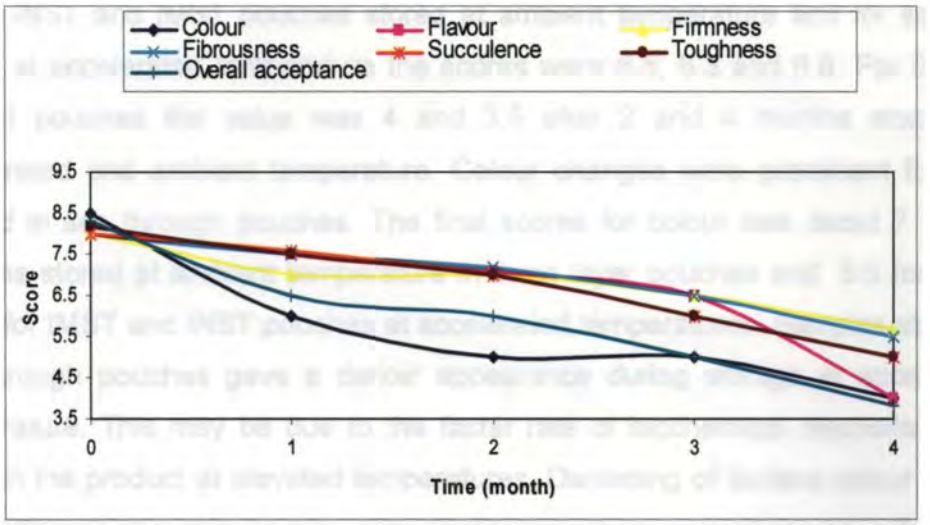


Fig. 87. Changes in sensory values of smoked and thermal processed tuna in brine in indigenous two layer pouches during storage at 28 ± 2°C.

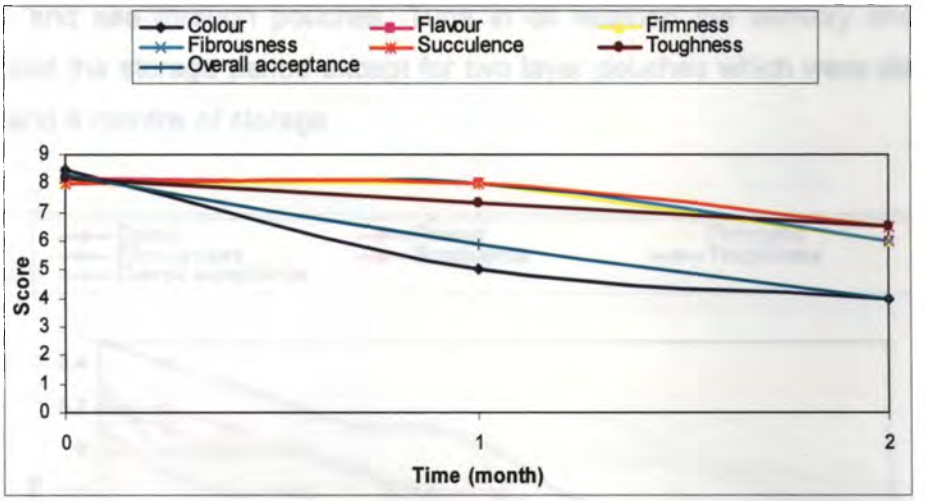


Fig. 88. Changes in sensory values of smoked and thermal processed tuna in brine in indigenous two layer pouches during storage at 37 ± 2°C.

4.6.6.2. Changes in overall sensory score of smoked tuna packed in oil

Changes in overall sensory score of tuna in oil stored in INOP, IMST, INST and INOP pouches at ambient and accelerated temperature are given in Figures 89- 96. The overall sensory scores at the end of storage were 7, 6.8 and 7 for

INOP, INST and IMST pouches stored at ambient temperature and for samples stored at accelerated temperature the scores were 6.8, 6.3 and 6.8. For the two layered pouches the value was 4 and 3.5 after 2 and 4 months storage at accelerated and ambient temperature. Colour changes were prominent for tuna packed in see through pouches. The final scores for colour was about 7 for the samples stored at ambient temperature in three layer pouches and 6.5 for INOP and 6 for IMST and INST pouches at accelerated temperatures. Samples stored in see through pouches gave a darker appearance during storage at accelerated temperature. This may be due to the faster rate of biochemical reactions taking place in the product at elevated temperatures. Darkening of surface colour after 1 month storage at ambient and accelerated temperatures was observed in the INTL pouches. The changes in flavour were not very prominent, final scores for flavour ranged from 7.5-7 for ambient and accelerated stored tuna. No significant taste difference was observed among the tuna products packed in the three layer opaque and see through pouches. Tuna in oil retained the sensory characters throughout the storage period except for two layer pouches which were discarded after 2 and 4 months of storage.

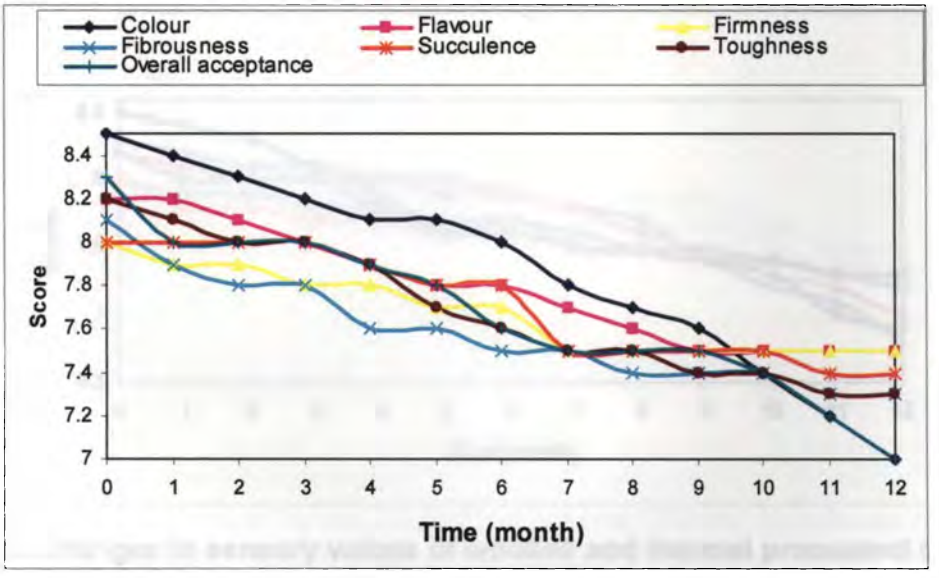


Fig. 89. Changes in sensory values of smoked and thermal processed tuna in oil in indigenous opaque pouches during storage at 28 ± 2°C.

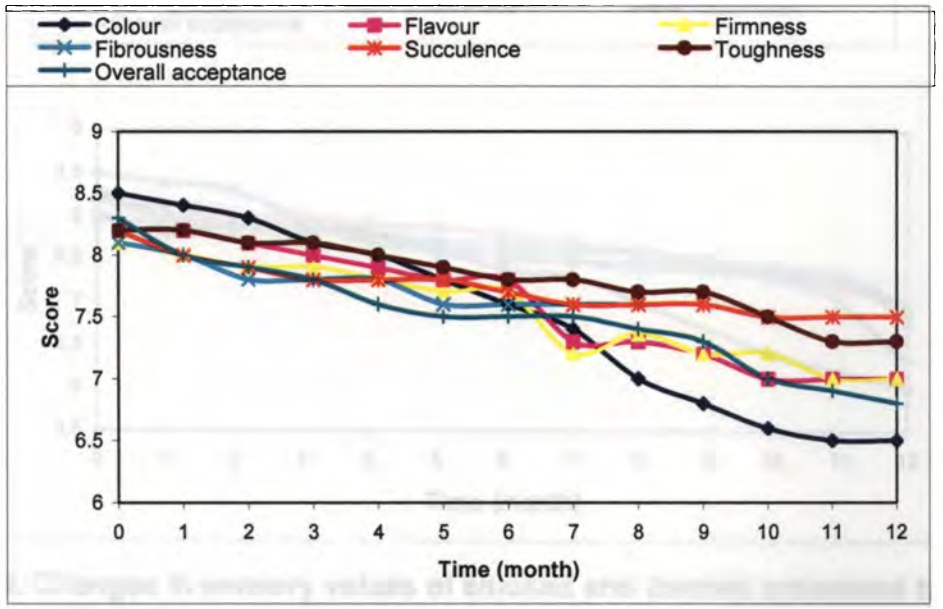


Fig. 90. Changes in sensory values of smoked and thermal processed tuna in oil in indigenous opaque pouches during storage at 37 ± 2°C.

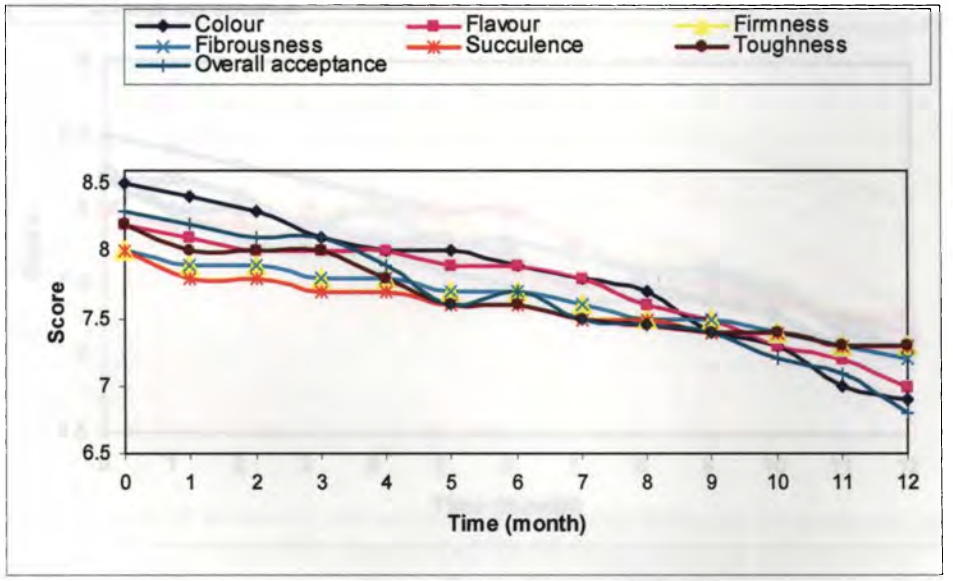


Fig. 91. Changes in sensory values of smoked and thermal processed tuna in oil in indigenous see through pouches during storage at 28 ± 2°C.

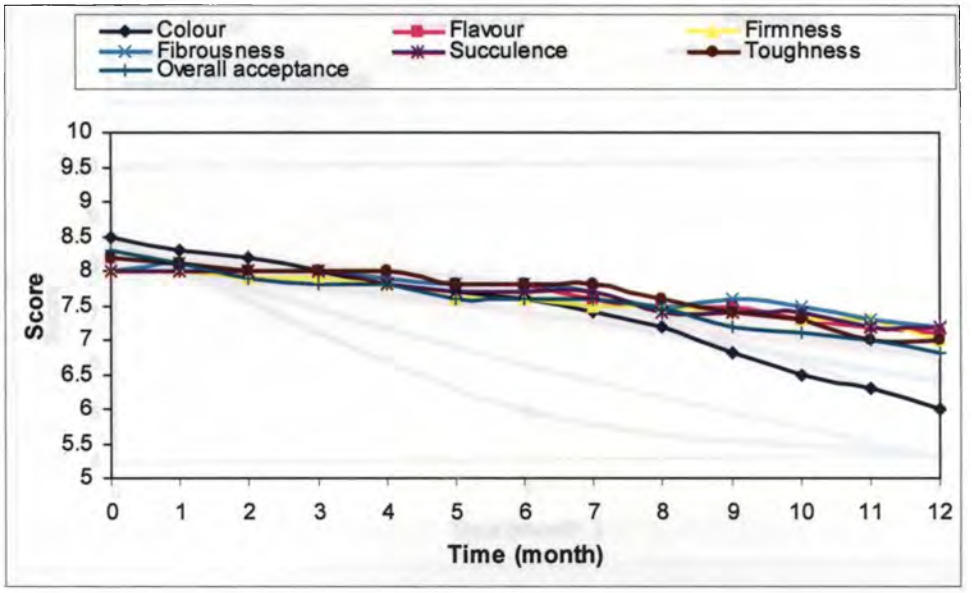


Fig. 94. Changes in sensory values of smoked and thermal processed tuna in oil in imported see through pouches during storage at 37 ± 2°C.

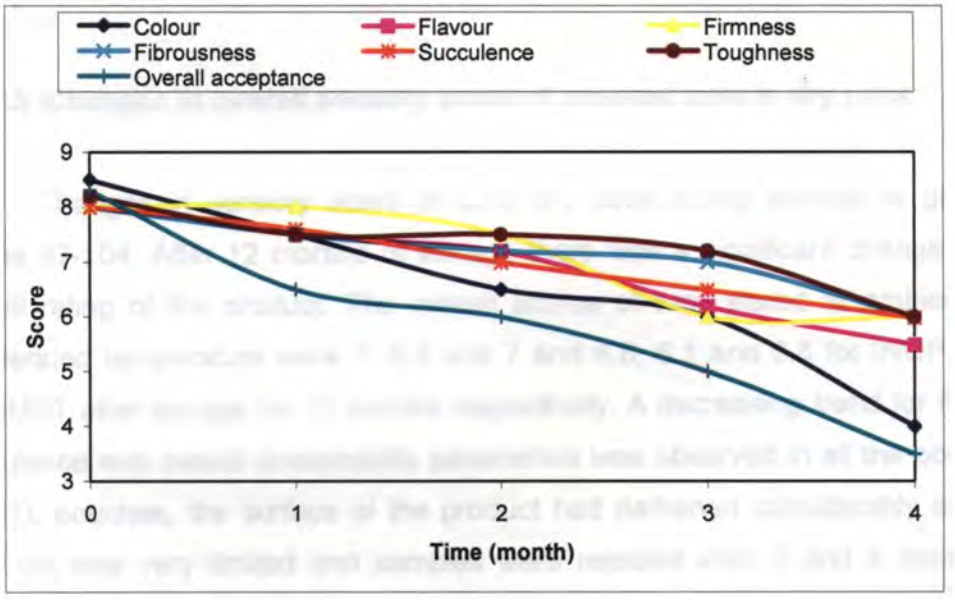


Fig. 95. Changes in sensory values of smoked and thermal processed tuna in oil in indigenous two layer pouches during storage at 28 ± 2°C.

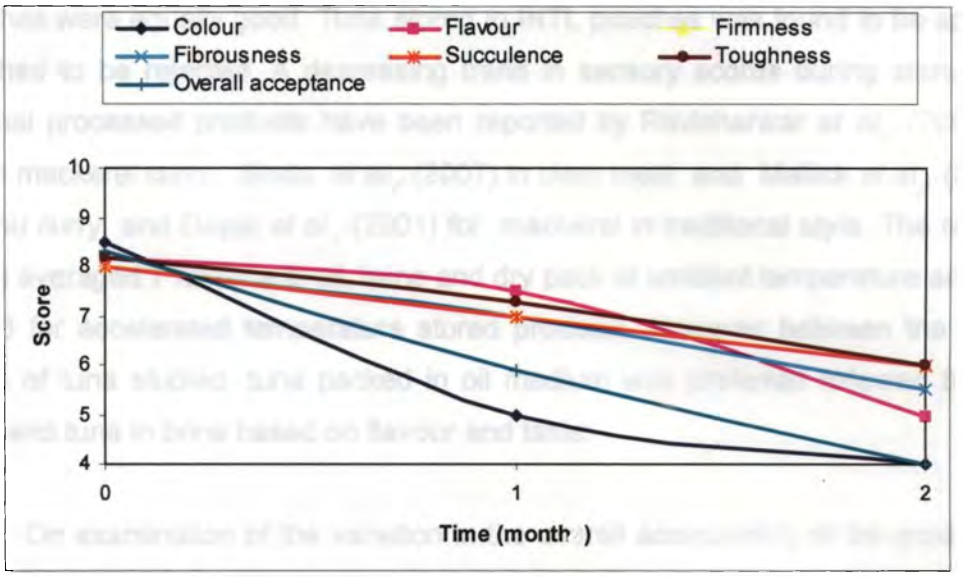


Fig. 96. Changes in sensory values of smoked and thermal processed tuna in oil in indigenous two layer pouches during storage at 37 ± 2°C.

4.6.6.3. Changes in overall sensory score of smoked tuna in dry pack

Changes in sensory score of tuna dry pack during storage is given in figures 97-104. After 12 months of storage there was a significant change in the overall rating of the product. The overall scores of tuna stored at ambient and accelerated temperature were 7, 6.9 and 7 and 6.8, 6.1 and 6.8 for INOP, INST and IMST after storage for 12 months respectively. A decreasing trend for flavour, succulence and overall acceptability parameters was observed in all the pouches. In INTL pouches, the surface of the product had darkened considerably and the shelf life was very limited and samples were rejected after 2 and 4 months of storage.

When the three different retort pouches were compared, it was observed that samples stored in INOP pouches were superior to the see through pouches. This is mainly due to the excellent barrier properties and presence of aluminum foil which do not allow the entry of light. Samples stored in three layer see through

pouches were equally good. Tuna stored in INTL pouches was found to be spoiled and had to be rejected. A decreasing trend in sensory scores during storage of thermal processed products have been reported by Ravishankar *et al.*, (2008) in Goan mackerel curry, Bindu *et al.*, (2007) in clam meat and Mallick *et al.*, (2006) in rohu curry and Gopal *et al.*, (2001) for mackerel in traditional style. The overall rating averaged 7 for tuna in oil, brine and dry pack at ambient temperature and 6.1 to 6.8 for accelerated temperature stored products. However between the three forms of tuna studied, tuna packed in oil medium was preferred followed by dry pack and tuna in brine based on flavour and taste.

On examination of the variation in the overall acceptability of the product in different pouches using Tukey's test it was found that the products packed in INOP, INST and IMST were equally acceptable($p \leq .01$). The overall acceptability was found to be decreasing significantly during the storage period ($p \leq .01$).

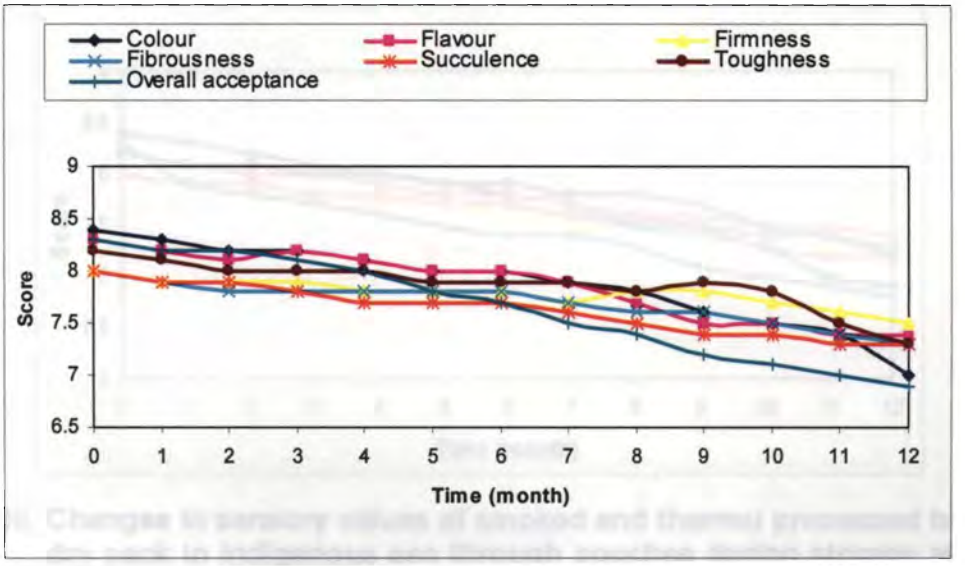


Fig. 97. Changes in sensory values of smoked and thermal processed tuna in dry pack in indigenous opaque pouches during storage at 28 ± 2°C.

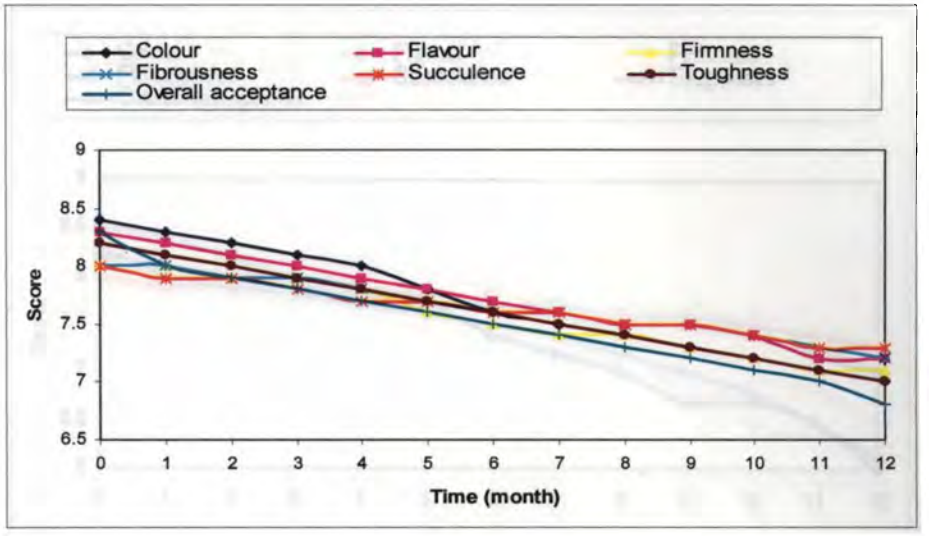


Fig. 98. Changes in sensory values of smoked and thermal processed tuna in dry pack in indigenous opaque pouches during storage at 37 ± 2°C.

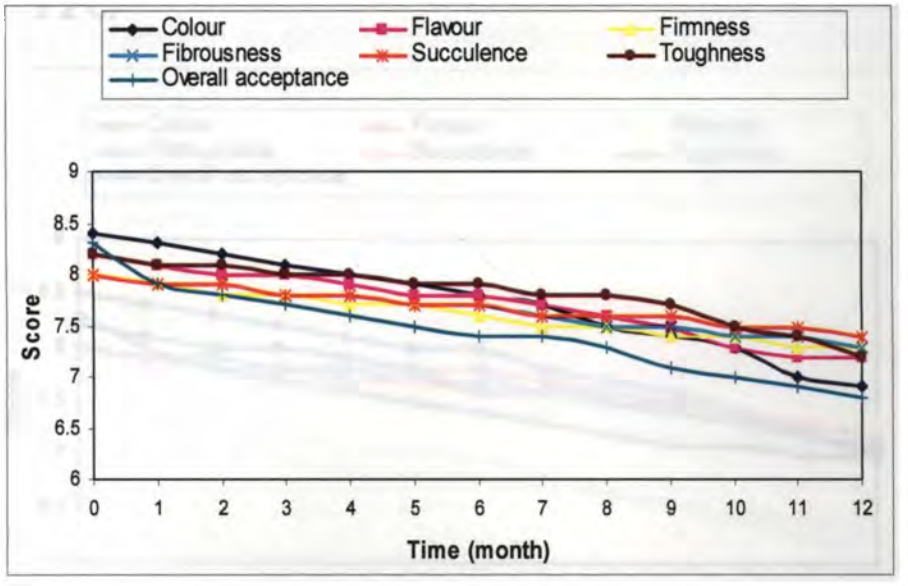


Fig. 99. Changes in sensory values of smoked and thermal processed tuna in dry pack in indigenous see through pouches during storage at 28 ± 2°C.

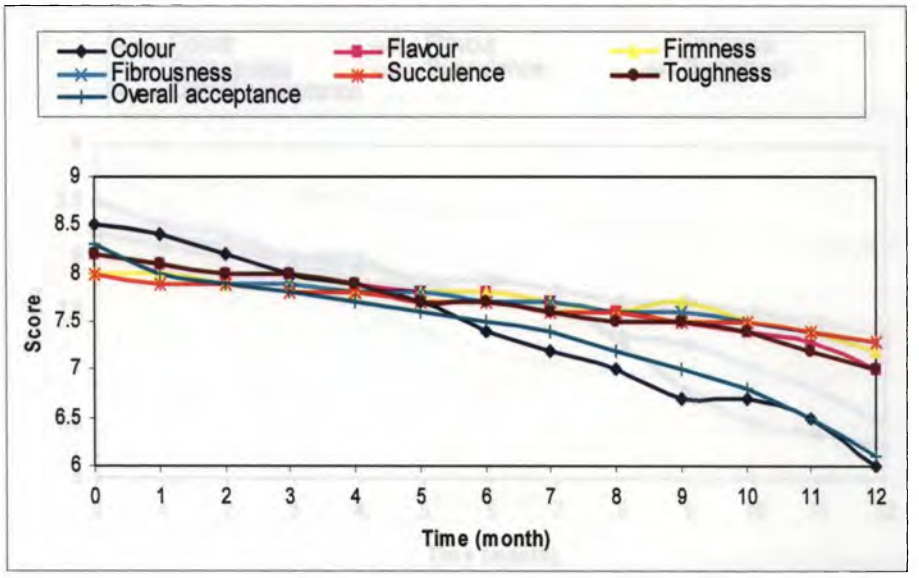


Fig. 100. Changes in sensory values of smoked and thermal processed tuna in dry pack in indigenous see through pouches during storage at 37 ± 2°C.

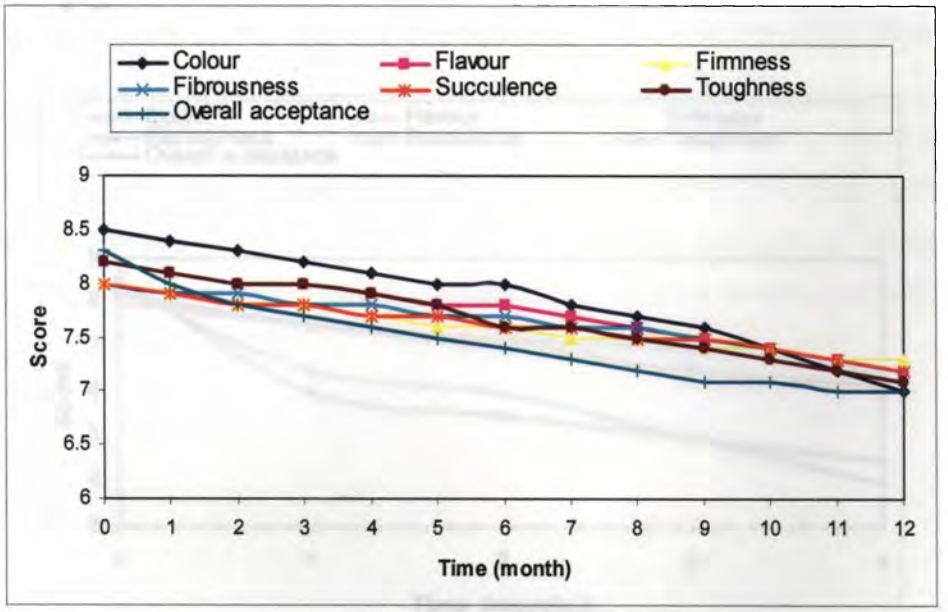


Fig. 101. Changes in sensory values of smoked and thermal processed tuna in dry pack in imported see through pouches during storage at 28 ± 2°C.

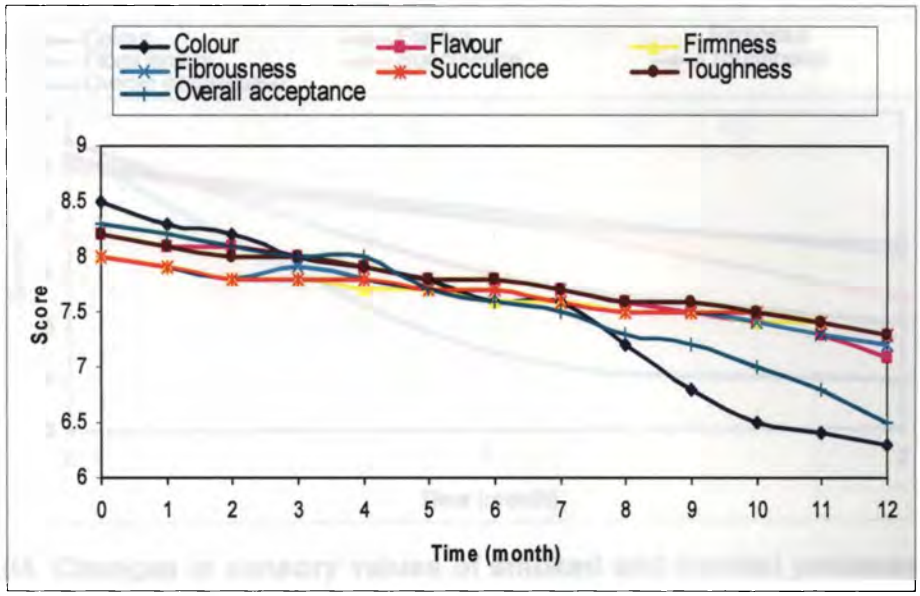


Fig. 102. Changes in sensory values of smoked and thermal processed tuna in dry pack in imported see through pouches during storage at 37 ± 2°C.

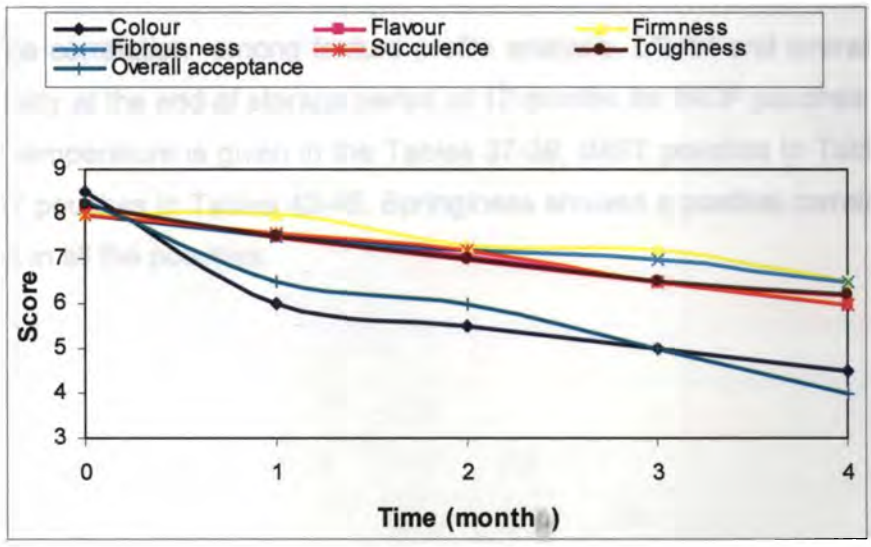


Fig. 103. Changes in sensory values of smoked and thermal processed tuna in dry pack in indigenous see through pouches during storage at 28 ± 2°C.

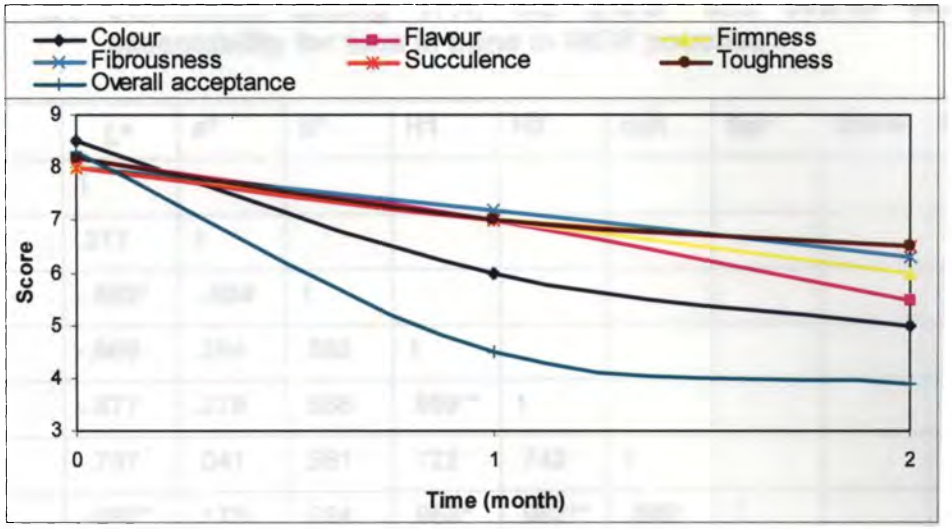


Fig. 104. Changes in sensory values of smoked and thermal processed tuna in dry pack in indigenous see through pouches during storage at 37 ± 2°C.

4.6.7. Relationship between the overall sensory acceptability, color and textural parameters at the end of storage period at ambient temperature.

The correlation among texture profile analysis, *L*a*b** and overall sensory acceptability at the end of storage period of 12 months for INOP pouches stored at ambient temperature is given in the Tables 37-39, IMST pouches in Tables 40-42 and INST pouches in Tables 43-45. Springiness showed a positive correlation with hardness in all the pouches.

Table 37: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna in brine in INOP pouches**

	*L**	*a**	*b**	H1	H2	coh	Spr	Chew	Overall
L*	1								
a*	.217	1							
b*	-.892*	-.634	1						
H1	-.869	.294	.552	1					
H2	-.877	.278	.566	.999**	1				
coh	-.737	.041	.561	.722	.742	1			
Spri	-.892*	.175	.624	.953*	.962**	.896*	1		
Chew	-.861	.304	.541	.999**	.997**	.688	.938*	1	
Overall	.914*	.208	-.817	-.776	-.794	-.869	-.892*	-.755	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).
H1- Hardness 1, H2- Hardness 2, Coh- Cohesiveness, Spr- Springiness, Che- Chewiness, and Overall- overall acceptance

Table 38: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna in oil in INOP pouches.**

	*L**	*a**	*b**	H1	H2	coh	Spr	Chew	Overall
L*	1								
a*	-.718	1							
b*	-.880*	.388	1						
H1	.969**	-.549	-.966**	1					
H2	.962**	-.522	-.936*	.990**	1				
coh	.303	.010	-.170	.306	.437	1			
Spri	.968**	-.553	-.970**	1.00**	.986**	.279	1		
Chew	.746	-.519	-.853	.788	.693	-.343	.805	1	
Overall	-.787	.859	.592	-.675	-.605	.257	-.687	-.799	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 39: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna dry pack in INOP pouches .**

	*L**	*a**	*b**	H1	H2	coh	Spr	Chew	Overall
*L**	1								
*a**	-.476	1							
*b**	-.020	-.869	1						
H1	.949	-.529	.056	1					
H2	-.240	-.204	.331	.078	1				
coh	.241	.158	-.279	-.077	-.998**	1			
Spri	.999**	-.436	-.064	.936*	-.279	.279	1		
Chew	-.885*	.540	-.100	-.987**	-.238	.238	-.866	1	
Overall	.803	-.765	.414	-.894*	.254	-.246	.772	-.991*	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 40: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna in brine in IMST pouches**

	*L**	*a**	*b**	H1	H2	coh	Spr	Chew	Overall
*L**	1								
*a**	1.000**	1							
*b**	-.972**	-.975**	1						
H1	.704	.696	-.531	1					
H2	.517	.509	-.354	.938*	1				
coh	-.575	-.574	.546	-.212	.139	1			
Spri	.691	.683	-.518	.999**	.949**	-.179	1		
Chew	-.819	-.811	.661	-.937*	-.758	.540	-.925*	1	
Overall	-.239	-.233	.109	-.740	-.927*	-.500	-.762	.458	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 41: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna in oil in IMST pouches**

	*L**	*a**	*b**	H1	H2	coh	Spr	Chew	Overall
*L**	1								
*a**	.769	1							
*b**	.608	-.040	1						
H1	.599	-.048	.996**	1					
H2	.622	-.062	.895*	.928*	1				
coh	.489	-.064	.845	.886*	.980**	1			
Spri	.567	.211	.622	.682	.905*	.904*	1		
Chew	.622	.154	.779	.826	.976**	.961**	.976**	1	
Overall	.001	-.014	-.003	.041	.197	.134	.359	.292	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 42: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna dry pack in IMST pouches .**

	*L**	*a**	*b**	H1	H2	coh	Spr	Chew	Overall
*L**	1								
*a**	.77**	1							
*b**	-.799**	-.85**	1						
H1	.84**	066**	-.776**	1					
H2	.88**	.75**	-.778**	.918**	1				
coh	.007	.004	.32**	-.25	-.10	1			
Spri	-.492*	-.37	.101	-.17	-.26	.586**	1		
Chew	.564**	.60**	-.68**	.496*	.543**	-.01	-.05	1	
Overall	.89**	.74**	-.82**	.85**	.841**	-.14	-.304	.588**	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 43: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna in brine in INST pouches**

	*L**	*a**	*b**	H1	H2	coh	Spr	Chew	Overall
*L**	1								
*a**	.949*	1							
*b**	-.717	-.463	1						
H1	-.250	.064	.854	1					
H2	-.683	-.427	.992**	.870	1				
coh	.502	.746	.236	.699	.256	1			
Spri	.074	.383	.640	.942*	.656	.896*	1		
Chew	-.974**	-.912*	.739	.305	.734	-.467	-.030	1	
Overall	.588	.642	-.298	.003	-.340	.487	.263	-.675	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 44: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna in oil in INST pouches.**

	L*	a*	b*	H1	H2	coh	Spr	Chew	Overall
L*	1								
a*	.555	1							
b*	-.198	-.925*	1						
H1	.287	-.635	.879*	1					
H2	.562	-.995**	.916*	.615	1				
coh	-.614	-.981**	.877	.552	.980**	1			
Spri	-.844	-.887*	.659	.224	.905*	.904*	1		
Chew	.244	-.668	.898*	.998**	.647	.594	.262	1	
Overall	-.857	-.639	.358	-.079	.675	.629	.896*	-.059	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 45: Correlation among TPA, CIE *L*a*b and overall sensory acceptability for tuna dry pack in INST pouches**

	L*	a*	b*	H1	H2	coh	Spr	Chew	Overall
L*	1								
a*	.780	1							
b*	.836	.995**	1						
H1	-.862	-.617	-.662	1					
H2	-.880*	-.500	-.564	.970**	1				
coh	.737	.947*	.950**	-.424	-.353	1			
Spri	-.314	.338	.252	.443	.621	.381	1		
Chew	-.935*	-.736	-.795	.628	.688	-.809	.220	1	
Overall	.791	.967**	.961**	-.754	-.620	.837	.227	-.659	1

* Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Summary and Conclusions

5.0 SUMMARY AND CONCLUSIONS

Product development and value addition is the need of the hour in the seafood processing industry. The consumer's demand for innovative and convenience foods are always on the increase. Buyers increasingly demand food products that are of high-quality, taste, appearance and nutrition and preferably, require minimum preparation time. Potential for ready to serve and ready to eat products in the export and internal trade is on the increase and a large variety of such products have flooded the markets. The affluent lifestyle and purchasing power made consumers choose ready to eat assembled meals than go in for raw food that has to be prepared and cooked before consumption. This trend is increasing with more and more business prospects in the food industry.

Retort Pouches are flexible, laminated containers that can be thermal processed like cans. The materials of these flexible containers can withstand thermal processing temperatures, provide superior barrier properties for a long shelf life and combines the advantages of both metal cans and plastic packages. Advantages of retort pouches over cans are many where the final processed product has a higher consumer appeal than those processed in cans due to the shorter process time and therefore a better quality retention and appearance.

High moisture smoked products are a delicacy in the advanced countries due to their sensory properties like flavour, texture and colour imparted during the smoking process. These highly priced products have to be stored at refrigerated temperatures and have a short shelf life. Smoked seafood that is vaccum packed and thermal processed in retort pouches and stored at ambient temperature had a shelf life of one year.

Fish and shellfish are highly perishable owing to their high water activity, pH and the presence of autolytic enzymes, which cause the rapid spoilage. They should be processed or preserved in the minimum shortest time without any delay.

Low temperature should be maintained from the time of harvesting, through handling up to the thermal processing stage. Tuna is one of the largest internationally traded fish commodity, which has contributed to 9% of the total trade in value terms. The Indian EEZ has rich potential and exploitation of tuna resources is one of the thrust areas for increasing marine products exports. There is an ever increasing demand for tuna products in the sashimi, chilled, frozen, canned and smoked forms worldwide.

It is only appropriate to develop an innovative and acceptable smoked product in the thermal processed ready to eat form in flexible pouches by using the local resources available in the country. This study was undertaken to develop a smoked product from yellowfin tuna with a high moisture content and capable of storage at ambient or room temperature with an extended shelf life. Locally available wood was standardized as the source of smoke flavour imparted to the fish. The smoked tuna was then thermal processed in flexible pouches and stored at ambient (28± 2°C) and accelerated temperature (37± 2°C) to determine the shelf life. Thermal processing was done in three different forms namely; tuna in brine, tuna in oil and tuna dry pack.

Indigenous see through pouches were made of three layers viz., outer polyester, middle nylon coated with silicon dioxide and inner of cast polypropylene. The silicon dioxide and aluminium oxide are nano particles capable of extending good barrier properties. The fourth pouch used in the study was a two layer see through pouch having layers of 10 µm outer polyester and 140 µm inside cast polypropylene. These pouches were tested for different physical properties to find their suitability for thermal processing. The thickness of different pouches ranged from 101 -118.8 µm for the three layer retort pouches and 131 µm for the two layered pouch. The tensile strength ranged from 450 kg/cm^2 in machine and cross direction for indigenous opaque pouches to 816 kg/cm^2 and 488 kg/cm^2 for the imported see through pouches. For the indigenous retort pouches it was 816 kg/cm^2 and 717 kg/cm^2 and for 2 layer pouches it was 316 kg/cm^2 and 292 kg/cm^2 respectively. Elongation at break was 20, 95, 78, and 53 for opaque, imported see

through, indigenous see through and two layer pouches. Heat seal strength given as percentage for the four pouches were 310, 504, 538 and 303 in machine direction and 237.9, 224, 412 and 286 in cross direction for opaque imported see through, indigenous see through and two layer pouches. The oxygen transmission rate (OTR) of the imported see through, indigenous see through and two layer pouches were 0.2 , 0.6, 2, and 5.5 $cc/m^2/24$ h at 1 atmosphere pressure at 21°C and the water vapour transmission rate (WVTR) were 0.2, 0.2, 0.86 and 1.99 $g/m^2/24$ h at 90 ± 2 % RH & 37°C. It was seen from the results that the migration into n-heptane stimulants was higher than distilled water in all the pouches and the values were very low and much below the limits of 10 mg/dm^2 or 60 ppm. The residual air in the thermal processed pouches was also below 2 % for all the four pouches. The pouches were all found suitable for thermal processing.

The raw tuna was of good quality and initial pH was 6.21 which decreased to 6.02 after brining and smoking. The total volatile base nitrogen (TVBN) content increased from 10.82 mg N/100g to 13.53 mg N/100g and the free fatty acid value (FFA) also increased from 5.54 in the raw to 6.89 after smoking. Thiobarbituric acid values which are compounds of secondary oxidation also showed an increasing trend from 0.23 mg malonaldehyde/kg in raw to 0.30 mg malonaldehyde/kg in smoked tuna. The levels of histamine in the raw tuna sample were 4.67 ppm which increased to 5.89 ppm after smoking. The levels of cadaverine and putrescence were very low in the raw (0.23 and 0.10 ppm), brined (0.36 and 0.05ppm) and smoked tuna (0.26 and 0.17 ppm). This biogenic amine valueincreased during the smoking process to 3.23 ppm. The level of spermidine in raw tuna was 0.16 ppm which remained the same after smoking the fish. However in the case of spermine the level decreased from 0.22 ppm in raw to 0.13 ppm in smoked. Tyramine levels in raw tuna were found to be 0.18 ppm which decreased to 0.15 ppm after smoking.

The individual PAH compounds in raw, brined and smoked tuna showed that Benzo-a-Pyrene was not detected in the raw and brine tuna taken in this

study. The tuna which was smoked with coconut husks at 75°C for a period of 60 min was found to contain 1.48 µg/kg of benzo-a-pyrene. Levels of other important polyaromatic compounds were dibenzo-anthracene (1.9µg/kg), benzo-a-anthracene (3.53 µg/kg), benzo-b- Fluoranthene, (2 µg/kg) benzo-k- fluoranthene (0.83 µg/kg) and indeno pyrene (1.9 µg/kg) and pyrene (9.2 µg/kg).

Carbonyl content was 0.14 mg/kg for the raw tuna. Tuna smoked with acacia and teak wood recorded the high value of 2.01 and 1.61 mg/kg followed by *cheruteak* and coconut husk which gave values of 1.41 and 1.01 mg/kg respectively. *maruthu, kolamavu, anjily* and *cashew* had low values of total carbonyls ranging from 0.42 mg/kg to 0.27 mg/kg.

Phenol was not detected in the raw and brine sample. In smoking tuna the phenol content was 0.74 mg/kg. Coconut husk had the highest phenol content of 0.62 mg/kg and cashew had the lowest with 0.31 mg/kg. In tuna smoked with *cheruteak* it was 0.49 mg/kg, *Kolamavu* 0.52 mg/kg, *Acacia* 0.32 mg/kg, *maruthu* 0.42 mg/kg, *anjily* 0.39 mg/kg and teak 0.56 mg/kg.

Smoking parameters were standardized based on the sensory scores, polyaromatic, carbonyl and phenolic compounds present in the wood and the availability and cost of the wood. The smoked tuna was then subjected to thermal processing in different pouches in three different forms using filling medium like oil, brine and as dry pack (without any filling medium). Four different types of flexible pouches were used for the thermal processing and storage of smoked tuna. Thermal process evaluation and heat penetration characteristics and changes during storage in various biochemical factors and smoke components were determined at regular intervals at ambient (28 ± 2°C) and accelerated (37 ± 2°C) temperature storage.

Smoked tuna was packed in four different pouches in three different forms and were subjected to heat sterilization in an over pressure autoclave and the data

was recorded. The total process time (T_B) and cook value (C_g) for smoked tuna in brine was 32 min and 75.61 min in INOP, 27.77 min and 69.74 min in IMST pouches, 31.91min and 77.98 min in INST pouches and 33.21 min and 74.14 min in INTL pouches respectively. In the case of smoked tuna in oil packed in INOP TB was 38.02 min and C_g was 86.83. In IMST pouches it was 29.25 min and 75.37 min, 33.25 min and 80.26 min in INST pouches and 36.35 and 79.82 min in INTL pouches. For dry pack tuna the T_B values were 42.01, 31.83, 38.04 and 39.1 min and the cook value were 89.31,77, 84.05 and 88.45 min for tuna processed in INOP, IMST, INST and INTL pouches respectively. All the pouches were found to be commercially sterile.

Effects of rotation on heat penetration parameters were studied. The total process time (T_B) in indigenous opaque pouches for smoked tuna in oil processed in a stationary retort to a Fo value of 10 was 38.02 minutes. T_B values for 2 rpm was 31.73 min, 31.11 min for 4 rpm, 30.05 min for 6 rpm and 28.92 min for 8 rpm. For smoked tuna in brine medium the T_B to reach the same Fo in a stationary retort was 32 min, whereas in the rotary retort it was 25.95 min at 2 rpm, 23.83 min at 4 rpm, 23.20 min at 6 rpm and 21.60 at 8 rpm. The data pertaining to smoked tuna in oil and brine subjected to stationary, 2 rpm, 4 rpm , 6 rpm and 8 rpm showed that total process time and cook value varied significantly between different levels of rotation.

The Changes in polyaromatic hydrocarbon components during storage of smoked and thermal processed tuna in brine, oil and dry pack showed that the initial BaP content for tuna in brine were 1.65 µg/kg which decreased slightly to 1.39 µg/kg after 12 months. Change in other important PAH compounds from initial to final were 0.81- 0.79 µg/kg for dibenzo-anthracene, 3.49 -3.12 µg/kg for benzo-a-anthracene,1.5 -1.08 µg/kg for benzo-b- fluoranthene, 0.55-0.35 µg/kg for benzo-k- fluoranthene, 7.40 - 4.61 µg/kg for indeno pyrene and 44.78 - 43.23 µg/k for pyrene. The BaP content of the smoked tuna in oil was 1.58 µg/kg which decreased to 1.13 µg/kg after storage. The changes in other PAH compounds of

importance like dibenzo-anthracene was 1.25 to 1.15 µg/kg, benzo-a-anthracene 3.51 - 3.80 µg/kg, benzo-b- fluoranthene 1.93 -1.50 µg/kg, benzo-k- Fluoranthene 0.23 -0.18µg/kg, indenopyrene 7.38 -36.30 µg/kg and pyrene 48.43 -34.73 µg/kg .The final content of BaP for tuna dry pack was 1.40 µg/kg , dibenzo-anthracene 1.40µg/kg, benzo-a-anthracene 3.53 µg/kg, benzo-b- fluoranthene 2.00 µg/kg, benzo-k- fluoranthene 0.83µg/kg , indeno pyrene 7.43 µg/kg, and pyrene 3.25 µg/kg.

The Changes in total carbonyl components during storage of smoked and thermal processed tuna in brine, oil and dry pack gave a decreasing trend. The total carbonyls in smoked tuna in brine have shown a decrease from 0.98 to 0.44 mg/kg in INOP, 0.89 - to 0.53 mg/kg in INST, 0.94 to 0.54 mg/kg in IMST and from 1.01 to 0.61 mg/kg in INTL pouches. The initial carbonyl values of tuna packed in oil medium were 0.93 mg /kg for INOP, 0.92 mg/kg in indigenous see through pouches and 0.95 mg /kg in IMST and 0.94 mg/kg in INTL. In IMST and INST pouches the values decreased to 0.63 mg/kg and 0.64 mg/kg respectively. In INTL pouches the carbonyl content was 0.67mg/kg after storage for 4 months. For smoked tuna dry pack the initial values of carbonyls were 0.85, 0.81, 0.80 and 0.85 mg/kg for INOP, IMST, INST and INTL pouches. The final levels after storage were 0.44, 0.66 and 0.63mg/kg in INOP, INST and IMST pouches. In INTL pouches the value decreased to 0.75 mg/kg after storage for four months.

The changes in total phenols during storage of smoked and thermal processed tuna in brine, oil and dry pack showed a decreasing trend. The initial total phenol values in INOP, IMST, INST and INTL were 0.80, 0.72, 0.83 and 0.76 mg/kg and final values were 0.22, 0.30 and 0.39 mg/kg for the opaque and see through pouches. The changes in phenol content of smoked tuna in oil medium decreased from 0.85 to 0.55 mg/kg in opaque pouches, 0.86 to 0.38 mg/kg in INST pouches and from 0.88 to 0.42 mg/kg in IMST pouches after storage for 12 months. In INTL pouches the values reduced from 0.89 to 0.72 mg/kg after storage for 4 months. Initial values for tuna packed in INOP, IMST, INST and INTL pouches

were 0.78, 0.70, 0.86, and 0.61 mg/ kg and final levels were 0.21, 0.20 and 0.15 mg/kg after 12 months of storage. The levels of phenols decreased to 0.40 mg/kg after four months in INTL pouches.

No significant decrease or loss of amino acids was observed between the raw, smoked and thermal processed tuna products. During storage certain amino acids were found to increase and decrease in some cases. This is mainly because of the variations in individual amino acid content. However the total percentage of amino acids remained the same.

The histamine levels of smoked tuna in brine decreased from 7.13 to 4.72 ppm. The initial and final level of putrescine was 0.38 and 0.31 ppm; cadaverine 0.26 and 21 ppm; spermidine 0.17 and 0.11 ppm; tyramine 0.27 and 0.17 ppm and agmatine 0.21 and 0.12 ppm. The level of histamine in tuna in oil was 6.93 ppm and decreased to 4.93 ppm after the storage period of 12 months. In oil packs final levels were putrescine (0.58 ppm), cadaverine (0.64ppm), spermine (0.11 ppm), spermidine (0.13ppm), tyramine (0.11ppm) and agmatine (0.13ppm). The final levels of histamine in the tuna dry pack was 6.43 ppm and others like putrescine, cadaverine, spermidine, spermine tyramine and agmatine were 0.17 , 0.2, 0.11, 0.17 , 0.19 and 0.19 ppm respectively.

Shelf life of the smoked and thermal processed tuna was evaluated during storage at accelerated (37±2 °C) and ambient temperature (28±2 °C).

The initial and final values of pH for tuna in brine in INOP pouches were 6.01 and 5.60, in IMST pouches 6.00 and 5.64 and in INST pouch 6.00 and 5.62. INTL pouches had a pH of 5.70 after 4 months of storage. In the case of accelerated storage the final pH values were 5.63 in INOP, 5.63 in IMST, 5.64 in INST and 5.72 in INTL pouches. The changes in pH for smoked tuna in oil showed a decreasing trend .The final pH levels were 5.6 in INOP, 5.62 in INST, 5.64 in IMST and 5.70 in INTL pouches. In the case of accelerated storage the final values were similar to that of ambient temperature stored tuna. The changes in pH values

of tuna in dry pack varied from 6.00 to 5.50 with an average value of 5.8. During the period of storage there was a decrease in the pH values from 6.03 to a final value of 5.50.

The TBA values of smoked tuna packed in brine had initial value of 0.26 mg/kg which decreased to 0.18 mg malonoldehye/kg in INOP. In INST and IMST the values increased from 0.32 and 0.12 to 0.33 and 0.22 mg malonoldehye/kg respectively. In INTL the TBA values were 0.13 which increased to 0.68 mg malonoldehye/ kg after storage for 4 months at ambient temperature. TBA values for smoked tuna in oil medium ranged from initial values of 0.26 to 0.15 in INOP, 0.24 to 0.14 for IMST, 0.13 to 0.33 mg malonaldehye/kg in INST pouches during twelve months of storage. For INTL pouches the initial value was 0.21 which increased to 0.61 mg malonaldehye/ kg after storage for 4 months. Initial values for tuna dry pack in INOP, INST, IMST and INTL pouches were 0.12, 0.24, 0.13 mg and 0.21 mg malonoldehye/kg respectively and final values were 0.36, 0.28 and 0.71 mg and 0.78 malonoldehye/kg. Similar increase was observed in the case of pouches stored at accelerated temperature in all the pouches in different media.

The changes in FFA content of thermal processed smoked tuna in brine medium at ambient and accelerated temperature storage showed a gradual increase in the FFA values during the storage period of 12 months from 1.88 to 4.80 mg % in INOP, 1.94 to 5.96 mg% in INST pouches and 1.42 to 4.94 mg % of oleic acid in IMST. For INTL pouches an increase from 1.46 to 2.82 mg % of oleic acid within a period of 4 months was observed. The changes in FFA of smoked tuna in oil showed that the initial and final values were 1.68 and 5.7, 1.94 and 6.5, 1.23 and 16.3 and 2.17and 4.11 mg % of oleic acid for the INOP, INST, IMST and INTL pouches. The final values for tuna in dry pack were 6.3, 6.9, 6.89 and 4.89 mg % of oleic acid for INOP, INST, IMST and INTL pouches. A similar increasing trend in FFA values was observed for the pouches stored at accelerated temperature.

The CIE L^* a^* and b^* values of smoked tuna in brine, smoked tuna in oil and tuna dry pack showed that L^* values (lightness) decreased in the tuna muscle during the storage period of 12 months. There was a slight decrease in the a^* (redness) value and an increase in the b^* (yellowness) values during storage. For tuna packed in INOP pouches the decrease in L^* values was less in comparison to see through pouches. In the case of tuna packed in INTL pouches the decrease in L^* values were very high after 4 months of storage. The changes were more evident in the case of samples stored at accelerated temperature.

Changes in texture profile during storage of smoked tuna in brine, oil and dry pack observed that in all the three forms of tuna the hardness 1 and hardness 2, springiness and chewiness values were found to decrease during storage. The cohesiveness value remained more or less the same throughout the storage period. Between the storage temperatures also there was no much difference in the textural values.

Sensory score of tuna in brine after twelve months of storage at ambient and accelerated temperature were 7.00 and 6.2 in INOP, 6.8 and 6.0 in INST, 6.8 and 6 in IMST pouches. The values for INTL pouches were 4 and below after 4 and 2 months storage for ambient and accelerated temperature in the entire three media used in the study. Changes in overall sensory score of tuna in oil stored at ambient temperature were 7, 6.8 and 7 in INOP, IMST and INST pouches and 6.8, 6.3, 6.8 and 4 at accelerated temperature. For dry pack tuna the rating was 7, 6.9 and 7 in INOP, INST and IMST after storage for 12 months. In accelerated conditions final scores after 12 months were 6.8, 6.1, and 6.8 for INOP, IMST and INST pouches respectively.

The research findings can be summarized as follows

1. Developed a high moisture smoked tuna product capable of storage at ambient temperature with extended shelf life.

2. Standardised indigenously available wood as the source of wood smoke based on different parameters and coconut was found suitable based on cost, availability, flavour, colour and BaP content.
3. Smoking was found responsible for imparting the characteristic flavour and colour to the tuna product.
4. Indigenous opaque and see through three layered retortable pouches and Imported see through pouch were found suitable for thermal processing and storage of smoked tuna in oil, brine and dry pack.
5. Indigenous two layer pouches made of PEST/CPP were found suitable for thermal processing based on physical properties but unsuitable for storage of thermal processed products
6. Heat penetration rate was faster in brine medium followed by oil and dry pack.
7. Heat penetration rate was quicker in see through three layer pouches compared to opaque retortable pouches
8. Rotation (2 rpm, 4 rpm, 6 rpm and 8 rpm) increased the heat penetration rate and decreased the total process time. Time to reach Fo 10 for smoked tuna was quicker at higher rpm compared to stationary retort.
9. Smoked and thermal processed tuna in oil medium was found to be more favored organoleptically than tuna in brine and tuna dry pack.
10. Smoke components like PAH remained unchanged during storage in dry pack and showed some decrease in the case of smoked tuna in oil and brine
11. Total carbonyl values decreased during storage. Browning of the filling media was seen in the case of tuna in brine and oil.
12. Total phenols also showed a decreasing trend during storage of smoked tuna in different media.
13. Changes in colour and sensory properties were more prominent in tuna packed in see through pouches.
14. Tuna stored at accelerated temperature had a lower organoleptic score compared to ambient temperature stored products.

15. Tuna packed in different three layer retortable pouches and stored at ambient and accelerated temperature were acceptable after a period of one year storage

This study is the first of its kind in India, where in smoked and thermal processed products have been developed using locally available wood as the source of wood smoke and flavoring and a shelf life of one year has been achieved. Retortable pouches of three layers, both imported and indigenous were found suitable to store thermal processed products. Heat penetration rate is quicker in retort pouches due to their thin profile in comparison to cans and hence the total process time is lesser. The nutritional and sensory attributes of the pouch products are better retained during processing. Hence these products are more acceptable than canned products. Indian vegetarian food products and fish curry products are available in the ready to eat form in the markets. Smoked and thermal processed products have not gained an entry to the market and hence this study will pave an opening for such products. Currently trade in tuna products from India is meager compared to the global trade. In India proper utilization of tuna resources is yet to be achieved due to the lack of infrastructure for handling and knowledge of value addition. The raw material cost is also less due to the poor quality of the fish when landed. Hence, the availability of such products will help in the trade of tuna products, improving the quality of raw material landing and ultimately realizing a better value to the fishermen and processors.

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