

**QUALITY OF DRIED AND SALTED BOMBAY DUCK  
AND RIBBON FISH IN SELECTED FISH LANDING  
CENTERS OF WEST BENGAL AND MEASURES TO  
IMPROVE THE SHELF-LIFE**

A Thesis

Submitted to the

West Bengal University of Animal and Fishery Sciences, in partial  
fulfillment of the requirements for the degree of

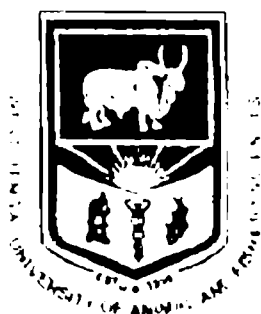
**MASTER OF FISHERY SCIENCE**

In

**Fish Processing Technology**

By

**TIRTHA BHATTACHARYA,**  
B.F.Sc.



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**CERTIFICATE**

This is to certify that the work recorded in the thesis entitled “Quality of dried and salted Bombay duck and Ribbon fish in selected fish landing centers of West Bengal and measures to improve the shelf-life” submitted by **Mr. Tirtha Bhattacharya**, in partial fulfillment of the requirements for **the Degree of Master of Fishery Science (Fish Processing Technology)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is a faithful and bonafide research work carried out under my supervision and guidance. The research findings presented in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

**Chairman  
Advisory Committee**

Date: 26.02.2015  
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# Approval Sheet


## Approval of the Examiners for the award of the degree of Master of Fishery Science (Fish Processing Technology)

We, the undersigned, have been satisfied with the performance of Mr. Tirtha Bhattacharya in the Viva-Voce Examination, conducted today, the .....28.02....., 2015 recommend that the thesis be accepted for the award of the Degree of **MASTER OF FISHERY SCIENCE** in Fish Processing Technology.

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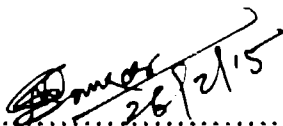
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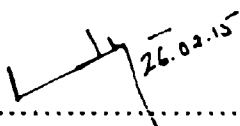
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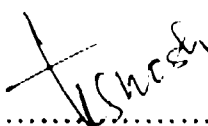
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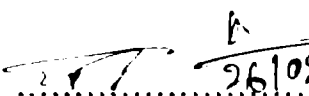
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Place: Kolkata

*Tirtha Bhattacharya*  
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## List of Abbreviations

(@)	At the rate of
<sup>o</sup> C	Degree Centigrade
DMRT	Duncan's Multiple Range Test
ANOVA	Analysis of Variance
FAO	Food and Agriculture Organisation
GDP	Gross Domestic Production
MPEDA	Marine Product Export Development Authority
BOBP	Bay of Bengal Programme
ICMSF	International Commission on Microbiological Specifications for Foods
FDA	Food and Drug Administration
EFSA	European Food Safety Authority
WHO	World Health Organisation
GRAS	Generally Recognised as Safe
PDA	Potato dextrose agar
AOAC	Association of Official Analytical Chemist
APHA	American Public Health Association
CFU	Colony forming units
cm	Centimeter
m	Meter
Fig.	Figure
gm	Gram
hrs	Hour
LDPE	Low density polyethylene
mg	Milligram
Min	Minute
PV	Peroxide value
RH	Relative humidity
SD	Standard deviation
TMA	Tri-methylamine
TMAO	Tri-methyl amine oxide
TVBN	Total Volatile Base Nitrogen
TBA	Thio-barbituric Acid
MDA	Malonaldehyde
TPC	Total plate count
TFC	Total Fungal Count
Kg	Kilogram
kms	Kilometers
JAB	Jalda khoti Bombay Duck
JAR	Jalda khoti Ribbon fish
JPB	Junput khoti Bombay Duck
JPR	Junput khoti Bombay Duck
CB	Control sample Bombay duck
T <sub>1</sub> B	T <sub>1</sub> sample Bombay duck

T <sub>2</sub> B	T <sub>2</sub> sample Bombay duck
CR	Control sample Ribbon fish
T <sub>1</sub> R	T <sub>1</sub> sample Ribbon fish
T <sub>2</sub> R	T <sub>2</sub> sample Ribbon fish
BD	Bombay Duck
RF	Ribbon Fish



*Chapter - 1*

**INTRODUCTION**

Fish is one of the most important sources of animal protein and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body (Ravichandran *et al.*, 2012). Fish and fishery products are highly nutritious and contain high percentage of animal protein with several other nutrients such as vitamins A, B, E and K and they are good source of some minerals like calcium, phosphorus and iron. It is a vital source of food for people. The FAO estimates that about one billion people world-wide rely on fish as their primary source of animal protein (FAO, 2000).

Fishery is an important sector and plays a vital role in the national economy-it provides full or part-time employment to 4.5 million people and contributes to food security of the country. With a coastline of over 8,000 km, an Exclusive Economic Zone (EEZ) of over 2.02 million sq km, and with extensive freshwater resources, fisheries play a vital role in the national economy and general livelihood of people. Presently, the inland fish production of our country has reached to 61.1 lakh tones with a growth rate of 7.3 % and marine fish production upto 34.30 lakh tones with a growth rate of 3.4 % (2013-2014) which makes India able to contribute a share of 5.43% of total global fish production. The fisheries and aquaculture sector contributes to about 1.0% of national GDP and 4.6 % of total agricultural GDP (Anon, 2014).

Marine Fisheries contributes to food security and provides direct employment to over 1.5 million fisher people besides others indirectly dependent on this sector. The bulk of the catch comprises of oil sardines followed by penaeid and non-penaeid shrimps, Indian mackerel, Bombay duck, Croakers, smaller quantity of cephalopods, other Sardines and Threadfin breams (CMFRI, 2009).

West Coast of India accounts for about 70% of the marine catch where bulk of the landings comprise of four major fisheries namely Oil sardine, Mackerel, Bombay duck and Penaeid and Non-penaeid prawns. However, elasmobranches, cat fishes, other sardines, Anchovies and White Baits, other clupeids, Sciaenids, Ribbon fish, Silverbellies and Penaeid prawns which constitute the bulk of the total catch of west Coast. On the other side, East coast accounts for about 30% of total landings while Andaman Nicobar group of Islands contribute 1% and Lakshadweep group of Islands 0.4%. The share of east coast for the landings of elasmobranches and marine catfish groups is more

than that of west coast. Pomfrets and ribbon fish are available more or less in equal quantity on both the coasts. Lakshadweep Island is important for skipjack tuna landings (Sen, 2005).

Preservation of fish by sun drying, salting, smoking and acid-curing or a combination of these methods forms the basis of preparation of traditional fish products of many countries. Asia is by far the largest producer of cured fish products. Amongst the countries producing dry fish in Asia are China, Japan, Indonesia, Philippines, India and Korea. In south-eastern and Far East Asian countries which are situated east of India, salted product with or without sun drying are produced in addition to traditional fermented fish sauce and paste (Sen, 2005). Fish drying, which is often regarded as a traditional and primitive method of fish preservation, varies from species to species, region to region and their availability all over the country. Although drying is regarded as a traditional, even primitive method of fish preservation in many developed countries, it is still of vital importance in the less well developed regions of the world, and will remain so for a long time to come (Waterman, 1976). Salting of fish followed by drying is a simple processing technique and it yields a product with relatively long shelf life (Prakash *et al.*, 2011). The fish must be dried quickly and hygienically in good sunlight and moving air. This protects the fish from microbes, insects and dirt. On the global basis, 14% of the marine landings are processed by curing (Sanjeev and Surendran, 1996) and which is highest among the different ways of disposition next to consumption of fresh fish. The cured fishery products have good potential for internal market and exported to various South and South East Asian countries such as Sri Lanka, Hong Kong and Singapore (Patterson and Govindan, 2009). Cured fish is a concentrated food rich in protein with well-balanced amino acid content. This factor, coupled with its low production cost, makes cured fish ideal in fighting the problem of acute protein malnutrition in the poor, especially in rural areas (Gopakumar, 1994).

Besides drying, long term preservation of fish by salting may be regarded as one of the practical and economic possibilities, for fisherman living in remote areas. Several studies have been conducted on the standardization of salting procedure, in order to improve the quality and general acceptability as well as to minimise wastage and losses in production. Nutrition is an important influencing factor of fish product/seafood

consumption (Olsen, 2004; Ahamed, 2009). At present, people are aware about health and nutritional issues (Hossain, 2008) and concerned about the nutritional value of the food items for their household.

Bombay duck, pink perch, croakers, mackerel, soles, seer fish, silver bellies, ribbon- fish and anchovies are usually employed for curing in our country. The hilly regions of India, still depends on dry fish sources. Since, a well-organized cold chain is yet to be established, the interior regions still depend on cured fishery products for their supply of fish. Depending on the regional variation in taste and consumer preference, different types of cured products are popular in different parts of the country.

India is the second largest fish producing country with the contribution of 5.43% in global fish production and West Bengal has been able to secure the second position among all the states of India (FAO, 2012). West Bengal has a coastline of 157.5 km and covers mainly the districts of South and North 24 Parganas and Purba Medinipur (Dan, 1985). Fish is an important part of the regular diet and is a cheap source of protein for the peoples of West Bengal. About 78% of total fish catch is consumed in fresh condition, 6% is used as dry fish and rest is used as frozen fish (Ghorai *et al.*, 2014). Indian dry fish export contributes 8% of all form of fish exports and earned 754 crores during 2012-2013 (MPEDA, 2013). The nutritional quality of dried fish remains intact, sometimes retains higher quality standards compared to fresh fish (Faruque *et al.*, 2012).

In ontological and temporal terms the Khoti, in West Bengal is the fishing camp that comes up at the landing centre and manages it for the duration of the full fishing season – roughly from July to February every year. The Khoti is a community-based organisation that during the full fishing season manages and governs the fishing activity, settles disputes and tries to take care of the needs and grievances of its members – the fishers and fish-workers. The importance of studying the Khoti regime lies in the fact that it is the organisation through which the main fishing activity on the coast takes place.

The technology that is followed for the production of salted and sun dried fish is essentially the same that was followed earlier. The products have the same defects and are of quality as reported in the earlier days. Two reasons which essentially are of socio economic nature may be ascribed to this. Firstly, the production is carried out on a home scale or in cottage industry scale in scattered places by people with knowledge of

traditional practices only. The products are consumed mainly by the weaker section of the population of developing countries and any improvement in quality would add to the cost of the product. Only recently, there is a realisation that the market for this product may be expanded by including economically better of sections who are ready to pay for better quality. With the available information it is known about the poor condition/ quality of the product and therefore it was felt necessary to improve the quality by adding certain chemical preservatives which would help in providing better quality and also extending the shelf life (Sen, 2005).

A study was conducted with the following objectives so as to assess the quality and shelf-life of two commercially important marine species (i.e. *Harpodon nehereus* and *Lepturacanthus savala*) most commonly dried in these khoti areas.

**The objectives of this study are:**

1. To analyse the quality of dry fishes (*Harpodon nehereus* and *Lepturacanthus savala*) produced at Junput and Jalda khoti of East Midnapore.
2. Storage studies of dry fishes (*Harpodon nehereus* and *Lepturacanthus savala*) treated with different chemical preservatives to assess the shelf life.



*Chapter -2*

**REVIEW OF LITERATURE**

## 2. Review of Literature

Fish is one of the major sources of proteins, vitamins and minerals and it has essential nutrients required for supplementing both infants and adults diet (Abdullahi *et al.*, 2001). Fish is an extremely perishable food item and soon after death; it begins to spoil (Agbon *et al.*, 2002). In the healthy live fish, all the complex biochemical reactions are balanced and the fish flesh is sterile. After death however, irreversible change that results in fish spoilage begins to occur. The resultant effect is the decomposition of the fish (Akinola *et al.*, 2006). So it needs immediate preservation just after the catch to extend its shelf life as well as to make sure for its continuity in supply in good condition during offseason.

### 2. 1. Status of Dried Fishes in India:

Dry fish is low cost dietary protein source and used as a substitute on scarcity (Siddique *et al.*, 2011). Upto mid-sixties, marine cured fish products formed the major fraction of total seafood exported both by weight and monetary value. But in subsequent years, frozen seafood and to some extent, canned products started gaining ground and quantum of traditional (Salted and dried products) seafood products started declining (Sen, 2005). During the financial year 2012-13, export of marine products from India reached an all-time high of Rs.18, 856 crores. The dried items exported from India was to the tune of 53,721 tonnes in the year 2011-12, which has increased to 72,953 tonnes in the year 2013-14 showing a positive growth in quantity of 35.80%. In terms of value which was Rs.562.63 crores in the year 2011-12, has increased to Rs.819.90 crores (2013-14) with an increase of 45.72% (MPEDA, 2013).

Till mid-fifties when fish preserving by canning and freezing had just started in India on a commercial scale, the traditional process was the principal method to preserve fish. Curing of fish is an ancient method of preservation in India that primarily involves two stages viz, salting and drying (Sanjeev and Surendran, 1996; Anon, 2001). In India, most of the marine landings are being processed into cured products. This is still applied to a large extent to preserve fish and squid (Sikorski *et al.*, 1995). Till 1959, about 50% of marine fish landed was utilised for curing. In subsequent years, share of cured fish started declining (Sen, 2005). At present, in India about 17% of the total catch is being used for salting and drying (Anon, 2001). The consumption of dried fishes is about 32% of the total marine landings in India and this rank second to fresh fish consumption (Thomas and Balachandran, 1989).

Salted and dried fish is a major source of animal protein available at cheaper price for the economically weaker sections of the society, especially people residing in coastal areas (Prasad *et al.*, 1999). India formerly was an exporter of dried prawn pulp and cured fish to countries such as Sri Lanka, Singapore, and Malaysia, and to some African and western countries (Gopakumar, 1994). About 8 million tons of fish (25-30%) of the world catch were being used for human consumption as dried, salted, smoked or treated by some combination of these processes (Kamruzzaman *et al.*, 1992).

In last few years, there has been no steady result in the export of cured sea food, but quantity of processed sea food is substantial and cured products still occupy an important position in the sea food export business of the country with about 8% of the total export of fish and fishery products of India (MPEDA, 2013). Certain sections of the consumers of the country have developed a preference for such products because of its characteristic taste and flavour. In addition, these products cater to the need of weaker sections of Indian people living in the inland areas including plantation labours and hill people (Sen, 2005).

## **2.2. Dry Fishes in West Bengal:**

### **2.2.1. Coastal Fisheries of West Bengal:**

India is the second largest fish producing country with the contribution of 5.43% in global fish production and West Bengal stands second among all the states of India (FAO, 2012). The coastal fishing community in India runs into millions. In West Bengal the number of people dependent for their livelihood on coastal fishing and allied activities add up to about two hundred thousand (Society for Direct Initiative for Social and Health Action, DISHA, 2013). West Bengal has a coastline of 157.5 km and covers mainly the districts of South and North 24 Parganas and Purba Medinipur (Dan, 1985).

Fish is an important part of the regular diet and is a cheap source of protein for the people of West Bengal (Ghorai *et al.*, 2014). The traditional coastal fishers of West Bengal are capture fishers who harvest the bounty of nature. In West Bengal Marine fisheries is mainly confined to the operation of bag nets, shore seines, stake nets and some seine nets. The fishing activities continue for three months in a year from mid-October to mid-January.

The marine resource base comprises 780 km<sup>2</sup> of inshore area (upto 20 m depth), 1815 km<sup>2</sup> (between 20 m and 80 m depth) and a continental shelf of 17,049 km<sup>2</sup> (upto 200 m depth) (BOBP, 1990). Brackish-water areas in the deltaic region encompass a territory of 200,000 ha. Marine fish landing in 2011-2012 exceeded 19.711 million tons (Handbook on Fisheries Statistics, 2011) for West Bengal, in which about half the catch comes from Purba Medinipur. Among this about 50% of the catch comes from the fishing activities of the traditional fishers. Pelagic fish forms 40% of the total catch, comprising Hilsa shad, Hairtail, Bombay duck, small clupeids and Engraulids. Among the demersals, Pomfret, Croaker and Catfish are the more important varieties. Pomfret, Hilsa shad and catfish are the dominant species in gillnet operations, though shark, ray and threadfin are also obtained. Ribbonfish, croaker, Bombay duck, prawn and small clupeids are caught mostly by set bag-net (BOBP, 1990).

#### 2.2.2. Status of dry fishes in West Bengal:

Marine fish drying is very common in the entire coastal zones of India. In West Bengal this practice is restricted to 24 Parganas and Purba Medinipur. These dried fishes have demand both in domestic and international market and plays an important role in employment generation of coastal poor people (Goswami *et al.*, 2002). About 78% of total fish catch is consumed in fresh condition, 6% is used as dry fish and rest is used as frozen. Indian dry fish export contributes 8% of all form of fish exports and earned 754crores during 2012-2013 (MPEDA, 2013).

A place called Egra (East Midnapore) is the largest dry fish market popularly called Balighai Dry Fish Market in West Bengal, which regulates the industry. Different kinds of dry fish from all processing areas of coastal West Bengal (Digha Mohona, Sankarpur, Jalda khoti, Saulaveri, Junput, Sagar Island, Tamplipuria) and Odisha usually come to this market. These dried fish later is supplied to different markets such as Assam, Siliguri, Odisha, Bihar and some other parts of India and abroad. The most common dry fish traded in this market are patia (*Lepturacanthus savala*), lahra (*Harpodon nehereus*), tapra (*Opisthopterus tardoore*), vola (*Pannamicrodon*), ruli (*Coilia dussumieri*), tauriboi, kagja, mourala, chanda etc (Ghorai *et al.*, 2014).

**FLOW OF MARINE DRIED FISH FROM WEST BENGAL: (BOBP, 1990)****Table No. 1. Depicting the flow of marine dried fishes from West Bengal.**

Serial No.	Dry Fish Markets	Supply Form	Consumption
1.	Territy Bazar	Behundi (Bag Net) operators in 24 Parganas.	Andhra Pradesh, Tripura, Nagaland, Kerala.
2.	Kerala Traders	As Above	Kerala
3.	Uluberia dry fish market	Behundi operators in Midnapore District.	Local and Calcutta retail
4.	Local Traders	Midnapore District	Orissa, Assam, Nagaland.

**2.2.3. Status of different Coastal Khotis of West Bengal:**

The term khoti is usually translated as 'fish landing centre'. It is a community of fishers and fish-workers coming together, from near and relatively far, to a particular place near the seashore in order to go about their business. The khoti brings together an entire host of people, fishers who take out boats and crew and catch fish. Sorters and driers; plus vendors and traders; and often shopkeepers, paramedics, and other people do business within the khoti area. The khoti is the fishing community's mode of civic and economic self-organisation; it is coming together by the fishers to ensure cooperation and manage conflicts.

There are about 78 numbers of khoti or fish landing community centre are present in West Bengal coast. Among these about 38 numbers of khotis are present in South 24 Pgs and 41 numbers in East Midnapore. This is to record that out of 41 numbers, about 39 khotis' continue to function, year after year, on the relatively short stretch of the East Midnapore coast and involving easily more than 50,000 fish workers, vendors, traders and their staff, and carrying on incredibly bustling activity. The bulk of fish that is caught in these khotis are dried. But in terms of the total fishing economics of the region under consideration, the revenue from raw fish constitutes not more than 15% of the total. About 85% of the gross revenue is from selling dried fish.

These dried fish from West Bengal coast that goes to other areas – the West Bengal hills, Assam and other states of the Northeast, to Jharkhand and Chhattisgarh.

Dried fish – which is cheap, keeps for months without going stale and without needing refrigeration – provides the poor with essential nutrients such as protein, B complex vitamins, particularly thiamine, Riboflavin, niacin, B<sub>6</sub> and B<sub>12</sub>, minerals such as iodine and phosphorus and other useful items (Society for Direct Initiative for Social and Health Action, DISHA, 2013).

According to the “*Asserting rights, defining responsibilities: Small-scale fishing communities and Coastal and fisheries management Perspectives in East Medinipur coast in West Bengal*”, a study made by Society for Direct Initiative for Social and Health Action, DISHA, 2013, the status of different Khotis in East Medinipore has been highlighted below:

### 2.2.3.1. Dadanpatrabar (Kharpai) Khoti:

As per oral tradition the Dadanpatrabar Khoti goes back to about 150 years. The total numbers of members involved in this Khoti is almost 4008, among them 3556 are males and 452 females. The members involved in this khoti, are occupationally divided as follows:

**Table No. 2. Depicting the number of people involved in different activities of Dadanpatrabar khoti.**

Category	Male	Female	Total
All	2576	1432	4008
fishing	1000	0	1000
Traders	30	0	30
Traders' Employee	270	0	270
Shorting, Drying, Cooking, Packing, Net Repairing	1080	1400	2480
Sundry activities	196	32	228

Currently there are about 150 boats in the Khoti, of which 82 were motorised and 68 Non-motorised. 95% of fishing activity is with the use of Behundi (Bag net) of mesh size 8 mm (cod end). And 5% is with the use of gill nets of mesh size 8-40 mm. There were 175 vendors, of whom 30 are female. But vendors are not khoti members.

### 2.2.3.2. Junput Khoti:

As per oral tradition the Junput Khoti goes back to the beginning of the colonial period or perhaps even earlier. The number of members in the Junput Khoti totalled 4800. Of these 1500 are female and 3300 male. About 1300 were involved in actual fishing and among these there were 120 layas. There are about 1800, of which the majority were males, were involved in sorting and drying. About 400 were large wholesale traders and about 700 were employees of these traders. The rest, about 600, were fish vendors who were members of the Khoti. The Behundi jaal overwhelmingly dominates fishing activity. One significant thing about Junput Khoti is that while women are mostly sorters and driers, a small percentage of women also indulge in 'from the shore' fishing, employing Kathi jaal and Shaula jaal.

### 2.2.3.3. Haripur Khoti:

This is a relatively new Khoti, fully functional from 1982. It was created by fishers belonging earlier to Junput and other large Khotis nearby. The fishers at Haripur say that for fishing purposes the Haripur beach is the finest in Purba Medinipur District. The total number of present members is 1150 of which 375 are women. 75% of the members are local – i.e. from Haripur and adjoining villages – and rest are from within 15 Km. The fishing activity involves are same as Junput khuti and the net used for fishing is mainly Bag Net, commonly named Behundi Jaal.

**Table No. 3. Depicting the number of people involved in different activities of Haripur khoti.**

Category	Male	Female	Total
All	775	375	1150
Fishing (Layas + crew members)	520	0	520
Sorting and Drying	125	375	500
Vendors	16	9	25
Other activities	80	25	105

### 2.3. Salting of Fishes:

In many developing countries where chilling and freezing facilities are often lacking, traditional curing methods are still the main method of preserving fish which cannot be sold fresh (Berhimpon *et al.*, 1990). Salting of fish has long worldwide traditions as means to preserve and increase the shelf life. In addition, it is a preliminary operation in some smoking, drying and marinating processes that have been mostly empirically developed and has remained unchanged for millennia. Salted fish products are popular in many countries around the world and considered safe for millenniums (Ismail and Wootton, 1992), even in developed countries (Turan *et al.*, 2007). Salting is generally aimed at reducing water activity ( $a_w$ ) to inhibit growth of spoilage microorganisms as well as inactive autolytic enzymes (Ashie *et al.*, 1996; Horner, 1997). Salt causes plasmolysis and alters protein and enzyme states in such a way that proteins become impervious to enzyme action and lose their efficacy. It also has bacteriostatic and bactericidal effects (Ismail and Wootton 1982). In order to achieve the product to be stable or safe to store at room temperature, a water activity of less than 0.85 is necessary, and can be achieved with 20% WPS (B.C. Centre for disease control, 2013). About 17% of the total catch goes for salt drying in India, of which the majority is from by-catch, excess catch and unmarketable varieties (Lakshmanan *et al.*, 2002).

Salting is one of the oldest methods of food preservation. Salting is mainly used to preserve products and also to promote important sensorial changes that make the final product appreciated by consumers (Andrés *et al.*, 2005b). It decreases the water activity and consists of transporting salt into food structures and is governed by various physical and chemical factors such as diffusion, osmosis and a series of complicated chemical and biochemical processes (Turan *et al.*, 2007). Preservation is based on lowering the "water pressure" or reducing the "usable" water for bacteria, toxins and mould spores, e.g. micro-organisms. When water is saturated with salt its usefulness is reduced so that only a few species of microorganisms can thrive (Bjarnason *et al.*, 1987). The effect of salt on bacteria is well known and it is fully proven that at high salt concentration (such as commonly used for fish salting) most putrefactive microorganisms of public health significance are not particularly tolerant whereas the salt tolerant microorganisms are less objectionable (Pizzali *et al.*, 1987).

In spite of new techniques and more recent (aseptic) preservation methods aimed to protect the delicate foodstuff which fish constitutes, salted fish remains a favourite food in those countries which do not scoff at salting in the absence of other methods of preservation (Bjarnason *et al.*, 1987). During salting, two main fluxes occur: the uptake of NaCl and other possible curing compounds, and the loss of water and some internal soluble solids (Chiralt *et al.*, 2001). Sodium chloride diffuses outside from muscles due to difference in osmotic pressure between the brine and fish muscle. This process does not continue indefinitely: sodium and chlorine ions form a water-binding complex with protein which itself exerts an osmotic pressure and eventually equilibrium is reached (Horner *et al.*, 1997). Solute diffuses from the salting agent and water diffuses out of the fish due to the difference in concentration and osmotic pressures between muscle cells and the salting agent (Barat, Rodri'guez-Barona, Andres and Fito, 2003). The salt uptake and water loss depend on the contact area and initial weight of fish (Fuentes *et al.*, 2007). The properties of fish muscle vary due to changes in water and salt content: the muscle gains salt, whereas water is lost or gained depending on the salting procedure (Thorarinsdottir *et al.*, 2002; Thorarinsdottir *et al.*, 2004 and Sannaveerappa *et al.*, 2004).

Salt uptake depends on many factors including species, muscle type, fish size, fillet thickness, weight, composition (lipid content and distribution), physiological state, salting method, and brine concentration, duration of salting step, fish-to-salt ratio, ambient temperature, and freezing and thawing (Wang *et al.*, 1998; Jittinandana *et al.*, 2002). The rate of the salt and water diffusion is positively correlated with increase in the brine concentration (Poernomo *et al.*, 1992; Bellagha *et al.*, 2007). Diffusion is said to be the most important mass transfer mechanism responsible for sodium and chloride transport. Solute diffuses from the salting agent into fish muscle and water diffuses out of the fish, due to the differences in osmotic pressures between inter-cells and salting agent (Raoult-Wack, 1994; Yao and Le Maguer, 1996). In addition, the presence of NaCl affects textural properties, water holding capacity, isoelectric point and protein functionality (Barat *et al.*, 2002; Duerr and Dyer, 1952; Dunajski *et al.*, 1979). The rate of salt uptake is very important with regard to weight change, water holding capacity (WHC) and quality of the final product.

### 2.3.1. Types of Salting:

Salting is usually performed by dry salting, brining, or injection of salt solution or a combination of these methods. The most commonly used methods by the industry are dry salting (pile) and brine salting (Boeri *et al.*, 1982, Birkeland and Bjerkgeng, 2005).

#### 2.3.1.1. Wet salting:

The cleaned fish are put in the previously prepared salt solution. The principle of wet salting is to keep the fish for a long time in a solution of salt and water, otherwise known as brine. Brining and pickle curing are the two methods used for wet salting. According to Berkel and Boogaard (2004), brining is not used as such as a preservation method but as pre-treatment for smoking or drying. With this method the fish is better protected against vermin and a more uniform salt distribution is achieved, brining requires the water used to be saturated with salt. To prepare the brine, mix four parts of clean water and one part of salt (for example, 10 litres (2 gallons) of clean, fresh water to 2.7 kg-3.6 kg (6-8 lbs of dry salt) in a clean, large plastic drum. Keep adding salt to the water, until no more salt will dissolve. In some fishes like seer, black pomfret, Indian salmon etc, the gut is removed and filled with salt in 1:3 proportions (Salting and Drying, South Pacific Commission, 1997).

Pickle curing entails preparing the fish as before. The fish are then laid alternately with dry salt in a water-tight container, such as a plastic container, using a fish-to-salt ratio of 1 part fish to 0.3 or 0.4 parts salt by weight. That is, for 10 kg of prepared fish you must use 3 to 4 kg of clean, dry salt. If less salt is used, then the fish will spoil. Water from the fish will quickly start to form. The surrounding salt will dissolve in this water. This is called the pickle. It is retained inside the container and will eventually cover all the fish.

#### 2.3.1.2. Dry salting (or Kench curing):

In this method, the fish is salted, but the juice and brine (pickle) are allowed to drain away. For 2 parts of fish, you need 1 part of salt. Layers of fish are separated by layers of salt and placed into a wooden box that has slight cut out on the sides, enabling the draining of juice. It is important to layer the fish with the first layer being flesh-side upwards, and the next layer of fish being flesh-side down. The final layer should be salt. Salting time varies from three days to a week, depending on the type and size of fish. In dry salting, the size of salt crystals is important. Fine crystals tend

to dissolve too quickly and are dragged down and drained, whereas large crystals dissolve very slowly and there is a risk of deterioration. Fine and larger crystals should be combined. (Salting and Drying, South Pacific Commission, 1997). Coarse salt is more suitable for dry (kench) salting. Fine salt will draw water too quickly from the outside of the fish, making the outside hard. As a result, the water inside the fish cannot escape and the salt cannot penetrate deep into the fish. Therefore, the fish spoils despite being salted. This is known as salt burn (Berkel and Boogaard, 2004). They also mentioned to use 30-35 kg of salt for 100 kg of clean fish. Use more salt where deep cuts have been made or the flesh is thicker.

Salted products can be classified into two groups, deeply salted and lightly salted, with different salting and consumption requirements (Chiralt and Fito, 2001). The former need to be desalted before consumption and their  $a_w$  value are close to 0.75, since they have aliquot phase saturated in NaCl. To reach the characteristics moisture and  $a_w$ , products are equilibrated with dry salt or submitted to sun or air drying before salting. The lightly salted products (ham, cheese, sausage, olives, pickles, etc.) are directly consumed.

#### 2.3.1.3. Mixed salting

In this process, simultaneous use of salt and brine is followed. The salting process is continued till the concentration of salt in the surrounding medium equalizes with the concentration of salt in the fish tissue. The salting process may affect the shape, structure and the mechanical features of muscle tissue.

#### 2.4. Drying of Fish:

Fish is a highly perishable food product and can be stored only by proper refrigeration or drying. Since most of the fishermen and gardener living at the coastal belt and hilly region are below the poverty line therefore refrigeration is distinct dream to them. The only alternative available is drying (Senadeera *et al.*, 2003), which is the most important techniques of food preservation (Menon and Muzumdar, 1987). Curing and drying are traditional low cost preservation techniques. The processing techniques vary with the type, nature, size and condition of the fish. Drying or dehydration is used to describe any process involving the removal of water from fish or fish product by evaporation (Eyo, 2001).

According to Balachadran (2001), drying method is considered as the least expensive for fish preservation. Drying along with salting and smoking as a fish preservation technique had been practiced perhaps longer than any other food preservation technique. Traditional methods which are almost exclusively based on marine fish and extensively followed in India are Sun-drying, Dry Salting and Mona Curing, Wet Salting, Pit- Curing, Colombo curing and smoking and drying. Drying is one of the most popular, primitive, low-cost and widely used fish preservation method (Sultana *et al.*, 2009). Salting alone does not allow long term preservation of fish and therefore most salted products are dried to bring down their water activity sufficiently low to have microbiological stability (Sen, 2005). Modern developments have centred on understanding and controlling the process to achieve the standard product demanded by today's market (Horner, 1992). It is still a vital method in the developing regions of the world. At present, about 1.4 million metric tonnes of fish which accounts for about 8.0% of the total world catch is cured and utilized. The important producers of cured fish are China, Japan, USSR, Indonesia, Philippines, Ghana, Canada and India (FAO, 2000).

According to Sharma *et al.*, (2009) solar drying is a potential decentralized thermal application of solar energy particularly in developing countries. Sun drying is a common farming and agricultural process in many countries, particularly where the outdoor temperature reaches 30°C or higher (Akarslan *et al.*, 2012). The drying of fish is a well-understood physical process. When a fish is exposed to air its surface begins to dry as moisture evaporates from the surface into the air by the process known as convective mass transfer. The rate of this evaporation is primarily governed by the humidity of the air stream, but is also affected by air speed. As the fish dries through the movement of the interior water to the surface, the moisture concentration gradient gradually decreases. Thus the driving force for the water transport decreases, and the drying rate slows. Drying will proceed at a progressively decreasing rate until equilibrium is reached, at which stage the fish is said to have reached 'equilibrium moisture content. With the completion on sun drying, fish meat becomes condensed, saturated with oil, becomes translucent and acquires an amber colour, a typical flavour, dense consistency and pleasant taste (Gerasimov and Antonova, 1979).

#### 2.4.1. Principle of Drying:

Drying is one of the oldest methods of food preservation in many communities of the world. The major principle of drying is focussed on reducing the moisture necessary for bacteria growth which can eventually cause deterioration of food. Compared with other methods of preserving fish and other food items, drying is regarded as the simplest method of preservation because the equipment used for drying is cheap, affordable and easy to manage (Ako *et al.*, 2004). It is necessary to dry the fish as quickly as possible from (45 to 60) % moisture content to 10% moisture or less (Ruiter, 1995). According to Silva and AndradeI, (2008) the water content in fish affects the microbiological and chemical stability, physical properties, processing, storage and distribution of fish. A safe moisture level of 6 to 8% in dried fish has been recommended in order to prevent spoilage due to microbe and pest proliferation (Effiong and Mohammed, 2008). However, the method of drying adopted by a fish processor will depend on the species of fish involved and the preference of the consumers. Drying depends upon the rate at which the moisture within the product moves to the surface by a diffusion process depending upon the type of the product. Besides this, Climatic conditions such as the temperature and relative humidity have great influence on sun drying.

#### 2.4.2. Types of Drying:

Method of drying fish varies between different countries and within the same countries depending on the type of fish used and the product desired.

Drying of fish is mainly carried out traditionally under open sun since the time immemorial. Sun drying represents a low cost processing technique to preserve fish. Sun drying of fishes is a traditional practice followed in many parts of the world (Sachithanathan *et al.*, 1985; N'Jai, 1985). This traditional method is followed for the preservation of fish especially in rural areas (Chakraborty and Verma, 1999). Open sun drying is widely practiced in tropical and subtropical countries to preserve agricultural products (Szulmayer *et al.*, 1971). It is abundant, inexhaustible, and environmental friendly (Basunia and Abe, 2001). According to Kolawole (2010), and Adeparusi (2003), exposure of freshly caught fish to heat from sunlight is often employed in the arid region of the tropics where the humidity is low and heat energy from sunlight is of considerable intensity and duration to dehydrate the fish before the

onset of spoilage. Sun drying is performed traditionally by placing fish on mats in the open (Tao *et al.*, 2008). Natural Sun drying can be improved considerably by raising the fish off the ground on wooden frames. This allows air to circulate beneath the fish, thus facilitating drying from both sides. It also breaks the cycle of insect reproduction (Relekar *et al.*, 2014). Waterman (1976) have reported that drying fish on racks with mosquito netting, wherein fish is more exposed to air and wind and less prone to contamination and insect infestation.

Alternative to sun drying involve the use of solar or artificial driers. Solar drying is often differentiated from “sun drying” by the use of equipment to collect the sun’s radiation for drying applications. There has been a great deal of research on the development of solar driers as an improved method of drying fish. Solar drying can be considered as an elaboration of sun drying and it is an efficient system of utilizing solar Energy (Bala *et al.*, 1998, Zaman *et al.*, 1989). Solar drier was developed by Chakroborty, (1976) where heat radiation from the sun is trapped by conduction process. Bala and Janjai have reported that, all the areas in the tropics and subtropics receive abundant of solar radiation and it is environmentally sound. Natural convection solar driers appear to have potential for adoption and application in the tropics and subtropics. The success achieved by indirect natural convection solar dryers has been limited, the drying rates achieved to date not having been very satisfactory (Oosthuizen *et al.*, 1996). These prompted researchers to develop forced convection solar dryers. i. e. (i) solar tunnel dryer, (ii) indirect forced convection solar dryer, (iii) greenhouse-type solar dryer, (iv) roof integrated solar dryer and (v) solar assisted dryer. Specialized dryers are normally designed with a specific product in mind and may include hybrid systems where other forms of energy are also used (Sharma *et al.*, 2009).

Several investigations has concluded that to meet the increasing demand for food preservation in developing countries, simple, cheap but efficient solar dryers, supplied with a fan for ventilation and supplementary heat sources should be developed (Arata *et al.*, 1991). Several designs of solar driers have been recently proposed for drying applications of fish in developing countries and still a good deal of work is continuing in this direction (Ratti, 1997). The Asian Institute of Technology (AIT) has developed packages on solar box dryer and solar-biomass hybrid dryers (Kumar *et al.*, 2005). Solar tunnel dryers have been tested with agricultural products such as bananas, chillies and radishes, and the performance is

found to be good as they considerably reduce the fluctuations in the drying air and solar radiation. But, there is limited information available on the drying kinetics of fish and prawn (Jain *et al.*, 2007).

The solar tent drier developed by Doe *et al.*, (1977) in Bangladesh, where solar radiation is allowed to pass through a clear surface which may be a transparent glass or polyethylene sheet and is absorbed by the black surface of the floor and the back of the drier. The use of a solar tent dryer for collecting and concentrating solar radiation to achieve elevated temperature during drying has been recommended as a good alternative to sun drying of fish (Doe *et al.*, 1977). Mohod and Sengar, (2011) have observed that during winter season, the average temperature inside solar tunnel dryer was found to be 50.39<sup>0</sup>C with corresponding relative humidity 11.92 % which was the lowest value and during summer season, the average temperature inside solar tunnel dryer was found to be 64.70 <sup>0</sup>C with corresponding relative humidity 10.67 %. Bala and Debnath (2012) have reported that the use of solar dryer leads to a considerable reduction of drying time in comparison to the sun drying, and the quality of the product dried in solar dryer was of good quality dried products as compared to sun dried products.

Mukherjee *et al.*, (1990) have developed a greenhouse type of solar fish drier of dimension 3mt X 2.5mt for fish drying which was further modified by Chakroborty (1995) based on the same principle. The processing techniques for dried aquatic products in the country is mainly conducted manually which is time consuming often resulting in low quality product and therefore mechanical driers were introduced.

With the availability of different driers namely, hot air drier, tunnel drier, fluidised bed drier, rotated drum drier which are capital intensive and requires heavy maintenance cost, the fisherman-processor families prefer the traditional method of drying fish over modern methods in order to produce large quantity dry fish at low cost. The products using this traditional method has the same defects and are of quality as reported in earlier days. Certainly it requires a good effort to introduce simple technological innovations at grass root level to provide better quality since these products are consumed mainly by weaker sections of the population of developing countries.

## 2.5. Biochemical aspect of Salted and Dried Fish:

### 2.5.1. Proximate Composition:

According to Relekar and Joshi, (2014), the proximate composition of fresh ribbon fish for moisture, protein, fat and ash were 75.66%, 17.66%, 2.08% and 0.76% respectively. They had also observed that initial moisture content of ribbon fish dried in Solar tunnel drier was 17.85%, raised bamboo platform 19.35%, black polythene sheet dried 19.95% and dried ribbon fish sample collected from market was 30.6% and same had increased at the end of 120 days storage. The ribbon fish dried in Solar tunnel drier recorded relatively higher protein content (44.13%) right from the beginning of storage, followed by fish dried on raised bamboo platform (42.22%). The dried fish procured from the market showed lower protein content (32.48%). Crude fat content of ribbon fish dried by different methods fluctuated in accordance with moisture during storage. The initial ash content of the dried ribbon fish procured from the market was lower (15.35%) as compared to dry on raised bamboo platform (16.73%).

Moisture content of seafood plays an important role in spoilage and lowering of moisture retards the spoilage (Stansby *et al.*, 1963). The seasonal variation in moisture content of dried seafood could be the reason for variable in drying time, environmental changes and level and type of salt used for curing (Anihouvi and Ayernorgs, 2006). Prakash and Jeyasanta (2011) have reported that the moisture content of sun dried sea foods of Tuticorin dry fish market had ranged from 30 to 55 % during monsoon and 15-25 % during summer season.

Increase in protein content was due to dehydration of water molecules present between the proteins causing aggregation of protein and these results in the increase in protein content of dried fishes (Ninawe and Rathnakumar, 2008). Siddique and Aktar (2011) have reported that protein level in the dried analysed (*Harpodon nehereus*, *Lepturacanthus savala*) samples varied from 51.98 to 58.33%, and 67.22 to 71.90% respectively during storage period, whereas the mean percentage of lipid of marine dry fish (*Harpodon nehereus* and *Lepturacanthus savala*) samples varied from 5.86 to 7.78% and 6.66 to 7.79% ; moisture level from 22.22 - 34.99% and 13.81 - 20.50% and ash level from 4.76 to 7.56%, and 4.64 to 4.86% respectively during storage period. Lovern (1950) has reported that the variation in lipid content was

influenced by the variation of species, diet, temperature, salinity, selective mobilization and distribution.

Pravakar and Mansur (2013) have reported that moisture content of dried ribbon fishes was  $23.94 \pm 0.2\%$  whereas in case of dried Bombay duck it was  $27.19 \pm 0.27\%$ . They observed that protein content of Bombay duck and ribbon fishes varied from 40.92 to 41.61% and 53.42 to 55.08 % and ash content varied from 19.64 to 20.31% and 10.29 to 11.64 % respectively. According to Siddique and Majumder (2012), the mean percentage of protein, lipid, ash and moisture of dried Bombay duck and ribbon fish varies from 52.44-71.32%, 4.92-8.64%, 4.68-6.72 % and 14.15-33.28 % respectively.

Islam and Ahamed (2010) have reported that the moisture content of dried fishes (*Puntius sp.*, *Amblypharyngodon mola*, *Channa punctatus* and *Glossogobius giuris*) obtained was in the range of 29.25 – 34.43%. On the other hand, protein content was found in the range of 32.02 to 41.38% with a huge variation in lipid content (3.21 to 14.03) %. Ash content was in the range of 20.14 -24.40 %.

## 2.5.2. Biochemical quality of Salted and Dried Fish:

### 2.5.2.1. Total volatile base nitrogen (TVBN) and Tri-methyl amine (TMA) :

TMA-N and TVB-N values indicate freshness of the fish (Beatty and Gibbons, 1936). Similarly, the production of TMA is dependent on the bacterial activity as well as from endogenous enzyme (Mohd. Yusuf and Sharif, 2010). The recommended level of the TMA-N value for human consumption is 10-15 mg/100 g (Connell, 1980). Prakash and Jeyasanta (2011) have studied the presence of spoilage indicators TMA-N and TVB-N in sun dried sea foods in different seasons of south east coast of India. The TVB-N values of the dried seafood during monsoon season were (18.4 -30 mg / 100g) followed by post-monsoon season (14.81-19.42 mg/100g) and summer season 14.14 mg/ 100g-9.31 mg/100g respectively, whereas TMA-N value during the monsoon season ranged between (7.1-16.34 mg/100 g) and the highest value recorded during post-monsoon season was 6.01-7.2 mg /100g. Bacterial putrefaction of spoilage bacteria is the reason for the sudden increase of TMA-N in fish muscle (Horie and Sekine, 1956). TVBN level in fish has also been used to indicate spoilage

and growth of microorganism (Lakshmanan *et al.*, 2002). The acceptable level of TVB-N in fishes is 35-40 mg /100 g (Kimura, 1934).

Accumulation of TMA-N and other amine compounds has been reported to occur during storage (Wu and Hambrick, 1982). Increase in TMA-N was also observed by Khuntia and Srikar (1990) during storage of dry salted mackerel. Recently Relekar and Joshi (2014) have worked on effect of improved drying methods on biochemical and microbiological quality of dried *L. savala*. They observed that the TVB-N content increased from 46 to 82.2 mg N% dried on raised bamboo platform, 89.5 to 247.5 mg N% dried on black polythene sheet and (70.6 to 211.56) mg N% for dried fishes procured from the market.

Immaculate *et al.*, (2013) have observed the quality and shelf life status of salted and sun dried fishes of Tuticorin fishing villages. The highest TVB-N values were observed in dry fishes during monsoon season and it ranged from 20.28 to 38.26 mg/100 g, where as in post monsoon season TVB-N values ranged from 15.77 – 22.72 mg/100 g and 9.90 – 19.20 mg/100 g in summer. Observation shows that TMA-N value during monsoon season ranged between 9.8 – 18.8 mg/100 g and 6.3 – 17.1 mg/100 g during post monsoon. TVB-N level of dried fishes in Tuticorin market was 30 -18.4, 18.95–14.81, 9.31–14.14 mg/100 g in monsoon, post monsoon and summer season respectively (Prakash *et al.*, 2011).

According to Pravakar and Mansur (2013), the total volatile base nitrogen (TVB-N) content of Bombay duck and Ribbon fish were (10.55 to 12.20) and (19.56 to 20.89) mg/100g respectively on dry weight basis. The lowest mean value obtained for Bombay duck was  $11.51 \pm 0.86$  mg/100g and the highest value for dried Ribbon fish was  $20.37 \pm 0.71$  mg/100g. Biochemical quality of dry salted fish products prepared from *Gudusia chapra* were observed during storage by Sharma and Kashyap (2013). During the storage period, total Volatile Base Nitrogen (TVBN) value for dry salted samples varied between 21.43 to 98.24 mg%, whereas for wet salted samples, it varied between 25.06 to 114.54 mg% and for unsalted samples from 32.48 to 148.30 mg%.

The biochemical and microbial quality of salted and sun dried seafood of Cuddalore coast were analysed by Saritha and Jayasantha (2012). The spoilage indicators such as TMA-N, TVB-N and FFA were above the acceptable limit. TVB-N measurement indicates the extent of the breakdown of protein due to bacterial and enzymatic action leading to amine production. Level of total volatile nitrogen in fish

is commonly used as a spoilage indicator (Pearson, 1976; Silva, 1998) and will help the microbes to proliferate for the production of total volatile nitrogen.

#### 2.5.2.2. Peroxide value (PV) and Thio - birbituric acid (TBA):

Fish lipid contains polyunsaturated fatty acids, which are highly susceptible to oxidation during prolonged storage. Lipid per-oxidation is one of the primary mechanisms of quality deterioration in stored foods, especially in muscle tissues (Gheisari, 2011). The changes in quality can be manifested by deterioration in flavour, colour, texture, and nutritive value and the production of toxic compounds (Pearson *et al.*, 1983). Relekar and Joshi (2014) have reported that peroxide value of dried ribbon fish increased throughout the storage, but the increase was only within narrow limits. The PV was more in case of fish dried on black Polythene sheet as compared to the other products.

Influence of ice storage for 13 days on raw material for the production of high quality dry fish products was studied by Reza *et al.*, (2006) and found that the TVBN, PV and TPC values up to 10 days of ice storage were within the acceptable limit, which increased beyond the acceptable limits at end of the storage period. The initial peroxide value was 1.27 mili equi O<sub>2</sub>/ kg fat which increased to the level of 21.4 m eq/kg oil in case of dry fish products.

An experiment was carried out by Kumar and Singh (2013) on changes in proximate, biochemical and microbiological characteristics of dried *Labeo gonius* fillets during storage at room temperature. The results of the experiment revealed that the fresh raw material had a peroxide value (PV) value of 19.54 millimoles of oxygen per kg of fat and free fatty acid content of 7.55% of total lipid as oleic acid and due to salting and drying, the value for PV increased to a maximum of 73.53 millimoles of O<sub>2</sub> per kg of fat after a period of three months storage. Connell (1976) has suggested that if PV is above 20 millimoles of oxygen per kg of fat, then the fish may show off odour and taste rancid. Bernardez (2005) stated that double bonds of unsaturated fatty acids are highly susceptible to oxidation and this leads to the production of carbonyls and other secondary oxidation products which impart the characteristic rancid off flavour to the product. Oxidation rancidity is most often measured by 2-thiobarbituric acid (TBA), peroxide value (PV) or carbonyl value (Gray, 1978). Comparison of TBA products of sample stored at room temperature showed that there is an increase in

TBARS substances for sample stored at room temperature than those stored at 15°C. This shows that the effect of temperature control reinforces the quality of dried stored fish. Low temperature storage results in a good product. Peroxide value of dried samples stored at room temperature varied from 23-54.26 miliequi O<sub>2</sub> / kg fat upto 8 months of storage period. Peroxide value of the control samples kept at 15°C was comparatively lower than that at 30°C (Sulochanan, 2012).

The increase in PV levels is probably due to the temperature of drying and the high content of unsaturated fatty acids. Shah *et al.*, (2009) have reported that peroxide value of *migaki-nishin* lipid increased rapidly from 5.52 to 11.86 miliequi/kg within 4 days of drying and then gradually increased up to 10 days of drying (16.07 miliequi/kg). Selmi *et al.*, (2010) have observed that the initial value of TBARS of dried silverside was 0.48 mg MA/kg oil, suggesting that lipid oxidation did not occurred during post-mortem handling to some extent. The experiment also revealed that, TBARS slightly increased within drying treatment to reach in solar and experimental dried silverside 1.27 and 0.87 mg MA/kg oil respectively.

Francisca (2010) has reported on proximate and organoleptic characteristics of sun and solar dried fish. He observed no much difference in the value of thiobarbituric acid in the sun and solar dried fish products. *G. niloticus* has the highest TBA value of  $1.06 \pm 0.03\%$ , while the lowest TBA value of  $0.67 \pm 0.12\%$  occurred in solar dried *H. niloticus*. Huss (1988) has reported that the acceptable limit of FFA in seafood is 0.5-1.5%. The Free fatty acid contents of all the seven salted and sun dried sea foods were high and were above the acceptable limit. TBA measured the deterioration in both extractible and non-extractible lipids and therefore had relevant application in the fatty foods especially fresh fish. Initial high TBA values in pre-processed products were reduced by the drying process. TBA is widely used as an indicator for the assessment of degree of secondary lipid oxidation. It has been suggested that a maximum TBA value, indicating the good quality of the fish is 5 mg malonaldehyde/kg while fish may be consumed up to a TBA value of 8 mg malonaldehyde (MA)/kg (Schormuller, 1969).

## 2.6. Microbial aspects in Salted and Dried fish :

Prakash and Jeyasanta (2011) have reported on microbial quality of salted and sun dried sea foods of Tuticorin dry fish market. The results of the experiment revealed that during monsoon season, the highest total plate count (TPC) of  $5.7 \times 10^4$  CFU/g was for *S. fimbriata*, where the lowest TPC ( $3.0 \times 10^4$  CFU/g) was observed in non-penaeid prawn *Acetes indicus*. During post monsoon season, the highest plate count ( $4.0 \times 10^4$  CFU/g) was observed in *S. fimbriata* and the lowest TPC ( $2.5 \times 10^4$  CFU/g) was noted in *A. indicus*, where fungal counts were high in sun dried sea foods during monsoon seasons. *S. fimbriata* had the highest fungal count ( $8 \times 10^3$  cfu/g) followed by *S. commerson* ( $5 \times 10^3$  cfu/g), *S. lysan* & *K. pelamis* ( $4 \times 10^3$  cfu/g), *S. commersonii* ( $3 \times 10^3$  cfu/g) and *A. indicus* ( $2 \times 10^3$  cfu/g). In post-monsoon period, fungal colonies were slightly decreased in all the sea foods where as in summer season again fungal counts were low. The quality of salted and sun dried fishes is adversely affected by the occurrence of fungi (CIFT, 1994). Also, the dominant fungi in salted and sun dried seafood vary with the place. The commonly occurring fungi in the west coast of India are *Aspergillus*, *Fusarium*, *Rhizopus* and *Mucor* (FDA, 1982).

In fresh fish, the acceptable limit of total bacterial count is  $5 \times 10^5$ /g at  $37^\circ\text{C}$  but for cooked or dried fish, the permissible limit is  $1 \times 10^5$ /g at  $37^\circ\text{C}$  (Surendran *et al.*, 2006). Experiment carried out by Kumar *et al.*, (2008) on dried fishes of Tuticorin market has recorded high bacterial count in *S. fimbriata* at  $3.5 \times 10^3$  CFUg<sup>-1</sup>. In Cochin market, the bacterial count in dried fishes was found to be less than  $10^7$  CFUg<sup>-1</sup> (Sanjeev *et al.*, 1997). The bacterial count of Nigerian fish market was observed by Adesiyan and Kaminjolo (1992) and the total bacterial count was found  $4.6 \times 10^6$  CFUg<sup>-1</sup> in dried fish sample.

Moisture content seems to be an exact indicator of the susceptibility of a product to undergo microbial spoilage (Troller, 1978). According to Lilabati and Vishwanath (1999), there is a direct relationship between the microbial count and moisture content of dry fish sample. Quality levels are based on the plate counts with representative sample unit not less than  $5 \times 10^5$  CFU/g for good quality, between  $10^5$ - $10^7$  marginally accepted quality and plate count  $10^7$  or above are considered unacceptable in quality (ICMSF, 1986-44 p).

Relekar and Joshi (2014) have reported that initial total plate count of ribbon fish dried in solar tunnel drier, raised bamboo platform, black polythene sheet and

market sample were  $2.96 \times 10^3$ ,  $3.80 \times 10^3$ ,  $1.12 \times 10^4$  and  $2.30 \times 10^4$  cfu/g respectively and the same increased to  $5 \times 10^3$ ,  $6.30 \times 10^3$ ,  $4.80 \times 10^4$  and  $5.80 \times 10^4$  cfu/g, respectively at the end of 120 days storage. Initially ribbon fish dried in solar tunnel drier and raised bamboo platform were observed to be free from fungus. However, TFC in solar tunnel drier and raised bamboo platform dried sample was recorded as  $4.60 \times 10^1$  and  $4.80 \times 10^1$  cfu/g respectively at the end of four months storage. The TFC of ribbon fish dried on BPS and market sample at initiation of storage was found to be  $0.9 \times 10^2$  and  $1.2 \times 10^3$  cfu/g, respectively, and then TFC in these samples increased to  $2.60 \times 10^2$  and  $2.70 \times 10^3$  cfu/g respectively after four months storage.

According to Kumar and Singh (2013), total plate count (TPC) of  $1.54 \times 10^4$  cfu/g was observed in dried *Labeo gonius* fillets stored at room temperature and the dominant genera of bacteria were *Pseudomonas* spp., *Micrococcus* spp., *Streptococcus* sp., *Bacillus* spp. and *Vibrio* spp. found in higher percentage. Islam and Ahamed *et al.*, (2013) have found that the Aerobic Plate Count (APC) was in the range of  $2.3 \times 10^5$ – $3.6 \times 10^7$  CFU/g in the dried fishes with the highest value from *Channa punctatus* and the lowest from *Puntius* sp. Pravakar and Mansur (2013) have found that the total aerobic plate count of dried Chinese pomfret, Bombay duck and Ribbon fish were  $3.8 \times 10^5$ ,  $3 \times 10^4$  and  $2.1 \times 10^5$  CFU/g respectively with lowest value in Bombay duck and highest value in Chinese pomfret. Reza *et al.*, (2006) have reported that the aerobic plate count of the iced stored dry fish was  $2.75 \times 10^3$  cfu/gm.

Saritha and Jayasanth (2012) have observed higher bacterial count of  $2.13 \times 10^6$  in sun dried fish (*Pomadys maculates*) found in cuddalore coast, and it had higher fungal count of  $2.2 \times 10^4$  where lower count was observed for *Acetes* sp. Chakrabarti and Varma, (1997) have reported that, *Aspergillus* sp. and *Mucor* sp. are the common fungi in the sun dried fishes of Visakhapatnam and Kakinada coast. Sharma (1989) has reported that *Aspergillus* sp., *Mucor* sp. and *Penicilium* sp. are known to cause food spoilage. Marine fungi in the dried fishes may have entered the fishes through the seawater used for washing or salt used for the preparation of brine (Shanthini *et al.*, 2003).

Presence of different types of fungi and bacteria in dried fishes has been reported by several authors (Gupta *et al.*, 1985, Philips *et al.*, 1976) and the moisture content supports the fungal growth. But the low moisture content of the samples adequately prevented the visible fungal growth. Reza (2006) has reported that aerobic plate count of 1 day raw material was in the range of  $2.75 \times 10^2$  cfu/ gm in silver Jew

fish to  $2 \times 10^3$  cfu/gm in Bombay duck. At the end of the 13 days ice storage, APC reached to  $10^7$ -  $10^9$  cfu/gm with minimum of  $2.3 \times 10^7$  cfu/gm in ribbon fish and maximum of  $4.3 \times 10^9$  cfu/gm in big eye tuna whereas the dried products of different fish species produced from 1 day iced stored samples had APC ranging from  $8.0 \times 10^2$  to  $5.4 \times 10^4$  cfu/ gm.

### 2.7. Organoleptic characteristics of dried fishes:

Haque and Kamruzzaman (2013) have observed the quality of solar dried and traditionally dried Bombay duck and Silver pomfret. The colour of solar dried Bombay duck and Silver pomfret fish became whitish to yellowish with little difference among fish species in traditional dryers. Texture was firm and flexible and colour was very natural in all samples. The overall quality of the products obtained from emergency solar tunnel dryer was excellent than traditional dryer for fish species. The colour of collected traditional sun dried fish samples were ranged from silvery to white with wide variations in different species and that of all the products were characteristics with firm and flexible texture. The overall quality of all the products from producer source was good though the sum of the colour score was not so attractive. The same products collected from wholesale market were of markedly deteriorative quality. They were stored for 5 to 7 months, which lead to deterioration in colour and texture compared to those of the original products. Slightly sour to sour odour was developed in many products.

Pravakar *et al.*, (2013) have observed the sensory quality of dried Chinese pomfret, Bombay duck and ribbon fish. The colour was lightly silver to whitish colour, which exhibited excellent colour for the dried fish products. Texture was firm and flexible and odour was very much natural in all samples. Insect infestation and broken pieces were not found in the products. It was observed that the flavour and colour are important factors influencing the overall consumer acceptance.

Khuntia and Srikar, (1990) have reported that dry salted mackerel with curing mixture had the best quality, followed by dry salted mackerel without curing mixture (sodium benzoate, potassium sorbate, sodium acid phosphate and butyl hydroxyl toluene at 0.25, 0.5, 1.5 and 0.25 % of salt weight respectively), wet salted product with curing mixture and wet salted product without curing mixture. Experiments carried out by the same authors revealed the better quality of salted pink perch with

additives, than those salted without additives, was depicted by its higher scores, throughout the storage period (Khuntia *et al.*, 1993).

### 2.8. Problems Associated with Dried Fishes:

Usually, fish drying is done by spreading the fishes on open beaches, roadsides, mats or even on the ground in open sun during. The open sun drying of fishes are associated with several problems such as contamination by dust and insect infestation leading to spoilage in non-monsoon period. This is mainly because of fishes are dried on mats spread on bare ground (BOSTID, 1988). There are several disadvantages encountered during open drying process, including contamination by dust, sand and stones, slow drying, blowfly infestation, attacks by rodents, ants, insects, poor handling and sanitation, improper processing which often lead to contamination and spoilage. One of the problems markedly evident is indiscriminate use of various types of insecticides. Sun dried fish treated with insecticides creates wide spectrum of health complications (Haque *et al.*, 2013).

### 2.9. Use of Chemical Preservatives:

Being the cheapest method of preservation of fish, the curing industry has not shown any noteworthy improvement from its primitive nature. The process and the requirements for products of improved quality and shelf life are now well known and are available to an entrepreneur for the last three to four decades or so. Yet even now the technology that is followed is essentially the same that was followed earlier. Besides observing strict hygienic practices at various stages of processing, several bactericidal and fungicidal chemical and antioxidants have been recommended to prevent/ retard the spoilage reactions.

For microbiological stability, a salt cured product should be dried sufficiently but such hard dried product may not have the acceptance of consumers who are accustomed to products with higher moisture content. Also, such hard drying which involves technological loss in weight would adversely affect the trade from commercial point of view. To obviate this difficulty, the use of chemical preservative or other similar means have been suggested from time to time. This would allow

retention of higher moisture content and the same time make the product comparatively stable (Sen, 2005).

Rao and Valson (1962) have reported that dip treatment of dressed mackerel in 4% propionic acid for 10 minutes prior to the use of salting and drying processes has been found very effective in controlling mould growth and reddening in the product upto 62 weeks compared to 15-20 weeks in the control.

Organic acids and their salts (acetic, lactic, propionic and sorbic) exerts antibacterial activity. They have been traditionally used as food preservative and are Generally Recognised as Substances (GRAS) approved as food additives by E.C, FAO/WHO and FDA (Surekha and Reddy, 2000). Sorbic acids and its salt have several advantages as food preservatives. Initially thought to have only anti-mycotic activity, they are now known to also inhibit a wide range of bacteria, particularly aerobic catalase – positive organisms. Potassium salt is commonly used because it is more stable. Furthermore, its greater solubility extends the use of sorbate to solutions appropriate for dipping and spraying (Thomas, 2000). Ahmed *et al.*, (2003) have reported that potassium sorbate has been extensively investigated as an antimicrobial agent for use in meat including fish to extend its shelf life and inhibit the growth of pathogens such as salmonella or *Staphylococcus aureus*.

Investigations by Sen and Rangaswami (1966) have revealed that the sun dried salted Indian mackerel prepared with curing salt mixture containing sodium benzoate and potassium sorbate was found to contain 142 mg % benzoic acid and 86% sorbic acid initially. After being desalted, the product had 59mg % benzoic acid and 38mg% sorbic acid. After six months of storage at ambient temperature (21-33)°C, the corresponding figures were 62 mg% and 27 mg% respectively. The above curing mixture is stable when stored at ambient temperature.

Wet salted pink perch, *Nemipterus japonicas* (Bloch), prepared using a curing mixture containing common salt, three preservatives (sodium benzoate, sodium sorbate and sodium di-hydrogen phosphate), and an antioxidant (butylated hydroxyl anisole), was found to have better keeping quality and longer shelf-life over those prepared using common salt alone. Food additives were found to effectively enhance the quality and extend the shelf-life of salted fish considerably. Further, the effect of

the additives was observed to be slightly more pronounced at ambient temperature ( $26.8 \pm 3.3^{\circ}\text{C}$ ) than at cooler storage temperature ( $2.5 \pm 1^{\circ}\text{C}$ ) (Khuntia *et al.*, 1993).

Fumigation of the product with burning sulphur or the use of a fish curing salt mixture containing 2% sodium acid phosphate and 0.25% sodium benzoate has been reported to be effective against the growth of red halophiles (Jarvis *et al.*, 1950). Investigations by Valsan *et al.*, (1961) with different preservative chemicals indicate that sodium benzoate along with sodium acid phosphate is effective in the prevention of development of red halophiles but is ineffective to prevent mould growth and sodium propionate or potassium sorbate prevents mould growth but occasional growth of red-halophiles is observed.

A curing mixture consisting of common salt, sodium benzoate, sodium acid phosphate, potassium sorbate and butylated hydroxyl anisole was developed by Sen *et al.*, (1967) and was found to extend the shelf life of salted pink perch (*Nemipterus japonicus*) and salted and sundried Indian mackerel (*Rastrelliger kanagurta*). The above curing mixture can also be used for salting and sun drying of other fishes.

On the basis of further investigations carried out with dry and wet cured catfish (*Arius spp.*), shark (*Carcharhinus spp.*) and black pomfret (*Parastromateus niger*), Valsan (1985) suggested the use of calcium propionate in place of sodium propionate. Calcium salt was found to be not only economical but also more effective. Direct dusting with the calcium salt (0.3g powder per 100g cured fish) was found to be most effective. There was no visible fungus attack or attack by red halophiles and no discolouration or off-flavour during 1 year of storage at ambient tropical temperature.

Scientist of Central Institute of Fisheries Technology, India carried out extensive investigation on the use of sodium propionate in cured fishery products. Sodium propionate completely suppressed the growth of moulds in cured fishery. The chemical was also successfully used in pickled fishery products (Suryanarayan *et al.*, 1962; Valsan *et al.*, 1963). Valsan, (1974) has recommended that refined common salt containing 3% sodium propionate should be dusted in the ratio of 1:10 (salt mixture: product) on the finished product just before its final packing and storing. Packing in a good container lined inside with polyethylene sheet was suggested to prevent further exposure to contamination and also to prevent excessive change in moisture content.

According to European Food Safety Authority (EFSA), the maximum safe level of propionic acid for poultry is 10 g/kg in complete feed. The corresponding safe concentrations in water for drinking would be 4 and 10 g/L. The inhibitory effect of different chemical food preservatives (Potassium meta-bi-sulphate, Sodium benzoate, Citric acid, Ascorbic acid and Potassium sorbate) on the growth of selected food borne pathogenic bacteria (*Staphylococcus aureus*, *Klebsiella aerogenus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) were investigated by Oladapo *et al.*, (2014), using agar diffusion technique. The concentrations used on test bacteria ranged from 0.125-1.5 mg/ml where the minimum inhibitory concentrations (MIC) of the five preservatives were 0.5, 1.5, 1.5, 1.5 and 1.5 mg/ml for potassium meta-bi-sulphate, sodium benzoate, citric acid, ascorbic acid and potassium sorbate respectively.

Experiments of qualitative and quantitative studies on micro-flora of low-salt herring slices supplemented with 0%, 0.2% and 0.3% of sodium benzoate (E-211) was done by Dabrowski *et al.*, (1998). It was observed that sodium benzoate reduced diversity of bacteria and yeasts in a tested product and exerted no influence on the total number of bacteria and yeasts. Ogiehor and Ikenebomeh (2004) have reported that sodium benzoate exerted high antimicrobial effects on the growth, survival and aflatoxin production ability of *A. niger*, *A. flavus* and *A. fumigatus*.

Arekemase and Salaudeen (2012) have experimented on effect of preservatives on proximate composition of tilapia (*Oreochromis niloticus*) and mackerel (*Scomber scombrus*) during refrigeration storage. This study was carried out to assess the proximate composition of Tilapia (*Oreochromis niloticus*) and Mackerel (*Scomber scombrus*) treated with varying concentration (1%, 3% and 5%) of sodium metabisulphite, sodium benzoate and potassium sorbate during 8-week storage at refrigerated temperature. Biochemical study showed a slight decrease in protein and lipid content while the moisture content and pH increased. This study shows that Potassium sorbate is more effective in preservation of the fishes than Sodium benzoate and Sodium meta-bisulphite at equal concentration.

Microbiological changes during processing and storage of salted-cured trout fillets treated with potassium sorbate were investigated by Oksuztepe *et al.*, (2010). For this purpose, 10 and 15% (w/w) NaCl and 1, 5 and 10% (w/v) potassium sorbate were applied to the fillets. Microbiological quality of all samples treated with 15%

NaCl and potassium sorbate were found better. Consequently, it can be concluded that the usage of potassium sorbate maybe useful and a synergistic effect between salt and potassium sorbate was determined.

Application of sorbates to foods includes direct addition in the formulation, spraying or immersion the food material in a solution, dusting with a powder or addition in coating or packaging material. Selection of the most appropriate method depends on processing procedure, types of food, objective to be accomplished, equipment available and convenience. In general, an amount of 0.1- 0.3% is tolerated, but levels as low as 0.1% may be detectable in some foods. Commonly the amount of sorbate used in smoked and salted fish were 0.05-0.3% (Safos and Busta, 1993).

The use of sorbic acid and sorbate are permitted in all countries for preservation of fish and fishery products as effective inhibitors of fungi. The dipping in 0.2 % potassium sorbate solution for 2 h was found to be sufficient for silver pomfret, goat fish and rainbow sardine to maintain residual sorbate level close to the accepted permissible limit of 1000 ppm in muscle (moisture level of 23 %). During storage at ambient temperature ( $28 \pm 5$  °C) with RH  $80 \pm 15$  %, the sorbate level in dried rainbow sardine and the value decreased rapidly to below 200 ppm in 6 months and visible fungal colonies appeared on these samples during the same period. Dried fish samples of Jew fish with lowest residual sorbate level i.e. 420 ppm, was free from visible fungal growth for 6 months. Residual sorbate level of above 200 ppm in the muscle of dried fish samples was found necessary to inhibit/delay the fungal growth during storage (Chakrabarti and Verma, 2009).



*Chapter - 3*

**MATERIALS AND METHODS**

### 3.1. Materials:

#### 3.1.1. Dry fish sample:

The samples of dry Bombay duck (*Harpodon nehereus*) and Ribbon fish (*Lepturacanthus savala*) were collected randomly from gunny bags from each substation (Khoti) of the study area. The samples were packed in sealed plastic polythene bags and brought to the laboratory of Department of Fish Processing Technology, Faculty of Fishery Sciences, and Kolkata on the same day.

#### 3.1.2. Fresh Fish sample:

Simultaneously, the same fish species (i.e. *Harpodon nehereus* and *Lepturacanthus savala*) were collected in fresh condition from the landing centre (Kakdwip) and brought them to the laboratory in iced condition (Fish: Ice = 3:1) using insulated boxes.

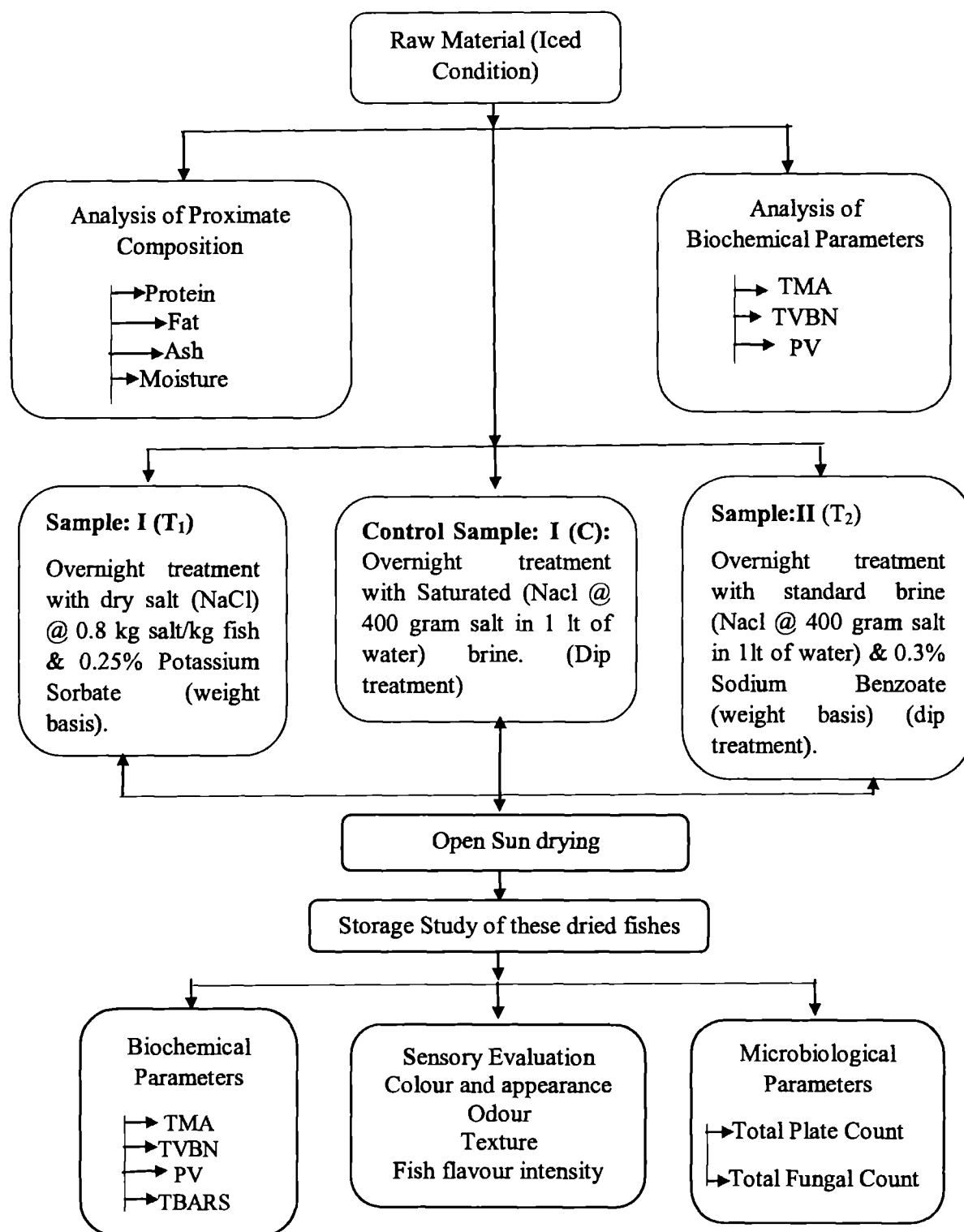
#### 3.1.3. Chemicals and glassware used:

Chemicals used for the analyses were of 'Analytical' reagent grades. The glasswares used were of 'Borosil' made. Total Plate Count (TPC) was estimated by using nutrient agar (NA) and mould count on potato dextrose agar (PDA) procured from 'Hi-media'.

#### 3.1.4. Equipments:

1. Hot air oven
2. Bacteriological incubator
3. Laboratory centrifuge
4. Autoclave
5. Electronic single pan balance
6. Laminar flow (vertical type)
7. Laboratory blender
8. Moisture balance
9. Homogenizer
10. Mortar pastel
11. Dessicator
12. VIS-UV Spectrophotometer
13. Kjeldahl distillation unit
14. Soxhlet apparatus
15. Muffle furnace

## 3.1.5. Experimental Design: (Fig No. 1)



## 3.2. Methods:

### 3.2.1. Preparation of sample:

Fishes were brought to the laboratory in iced condition and measured their length and weight. The fishes were washed properly and prepared for drying as per the method given in Fig. 1.

### 3.2.2. Drying of Fish:

The fishes were dried by following the same way of drying practised in those khotis. A bamboo structure was made in open sun and the fishes were kept hanging using hooks from the platform. During night time, the fishes were covered with polythene sheet in order to avoid uptake of moisture.

### 3.2.3. Packing:

After drying, the fishes were packed treatment-wise and also species wise in different low density polyethylene (LDPE) bags and tagged properly. Then these plastic bags were heat sealed and stored at room temperature for further storage study.

### 3.2.4. Nutritional, Biochemical and Microbial Analysis:

The fishes taken from the respective khotis (i.e. Jalda Khoti and Junput Khoti), were checked to know their nutritional quality by observing the proximate composition (Protein, Fat, Ash and Moisture), Biochemical (TMA, TVBN, PV, TBA) and Microbiological parameters (TPC/APC and TFC). The fresh fishes that were taken from fish landing centre, are undergo different biochemical analysis and proximate composition. During storage study, the sampling was done for the different biochemical and microbiological parameters at one month interval for a period of 4 months.

### 3.2.5. Methods of analysis during storage study:

#### 3.2.5.1. Proximate composition:

##### 3.2.5.1.1. Moisture Content:

The estimation of moisture content was done by following the method given by AOAC, (1984). In brief, 5 g of sample was weighed accurately in a pre weighed aluminium dish. The dish containing the sample was placed in a hot air oven without lid. The temperature of the oven was fixed at  $100\pm 5^{\circ}\text{C}$  and the samples were kept overnight (16 hours) for drying. The dish was taken out from the oven and

cooled in dessicators at room temperature. Total moisture content was estimated with the formula given bellow:

$$\text{Moisture (by \% weight)} = \frac{100 (W_1 - W_2)}{W_1 - W}$$

W = weight of the empty dish in grams.

W<sub>1</sub> = weight of the dish with the material before drying in grams.

W<sub>2</sub> = weight of the dish with the material after drying in grams.

#### 3.2.5.1.2. Ash content:

Estimation of ash content was done by following the method given by AOAC, (1984). In brief, 5 g of sample was weighed accurately in a porcelain crucible and dried in a hot air oven overnight at temperature of 100±5°C. Sample was char dried completely by heating over a burner and incinerated in a muffle furnace at a temperature of 550±50°C with adequate air supply until it became completely white. After that the crucible was taken out and cooled in a desiccator at room temperature. Total ash content was estimated using the formula given bellow:

$$\text{Total Ash (\% by weight)} = \frac{W_2 - W}{W_1 - W} \times 100$$

W = weight in grams of empty dish.

W<sub>1</sub> = weight in grams of dish with the material before incinerating.

W<sub>2</sub> = weight in grams the dish with the material after incinerating.

#### 3.2.5.1.3. Protein content:

Estimation of protein content of the sample was done by using Kjeldahl method (AOAC, 1995). In brief 1 g sample weighed in a dry Kjeldahl flask and about 5 g of digestion mixture and 20 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the digestion flask. Few glass beads were also added to avoid bumping. Mixtures was digested by heating, first slowly and then vigorously for 4-6 hours until the sample became clear and colourless to ensure complete conversion of nitrogen in the sample to ammonium sulphate. The flask was then cooled and the volume was made up to 250 ml by distilled water. 10 ml of 2% boric acid with 2-3 drops of Toshiro's indicator was taken in a 100 ml conical flask and placed in such a way that the tip of

the outlet of the condenser of distillation unit remained dipped into the boric acid solution. 5 ml of the digested sample was added to the previously cleaned the distillation chamber and about 10 ml of 40% NaOH was added followed by rinsing with distilled water. The steam distillation continued till about 30 ml distillate was collected (7-8 min) in the receiving flask. At the end the receiving flask lowered and hold for about 1 min at about 1 cm below the tip of the condenser. The condenser outlet washed into the receiving flask with distilled water. The boric acid turned green when ammonia was absorbed. A blank was also done through all the steps by taking distilled water in place of sample. The collected distillate was titrated against 0.02(N) H<sub>2</sub>SO<sub>4</sub> until the original red colour was obtained. Total protein content was estimated with the formula given below:

1 ml of 0.02 (N) H<sub>2</sub>SO<sub>4</sub> = 0.00028 g of nitrogen

$$\% \text{ Total nitrogen} = \frac{0.00028 \times \text{titre value} \times 250 \times 100}{5 \times \text{weight of sample}}$$

1 g nitrogen = 100/16 = 6.25 (conversion factor)

Hence;

% Protein = % nitrogen × 6.25 (conversion factor)

#### 3.2.5.1.4. Total fat content:

The fat content was estimated by Soxhlet method (AOAC, 1984). In brief, 5 g of sample was weighed in a thimble and plugged with cotton. The thimble was then fixed in to the apparatus. The fat was extracted with anhydrous ether (BP 40-60°C) for about 16 hours. After the extraction, the thimble was removed and the solvent from the receiving flask was collected out by distilling it off, before it returned into the flask by siphoning. Thus, maximum possible amount of solvent was restored. Finally, the traces of the solvent were removed from the flask by overnight drying it in oven at 100°C. After cooling the flask in the desiccators, the weight and the value was recorded. Final fat content estimated by using the formula given bellow:

$$\text{Fat content (g per 100g of sample)} = \frac{\text{Weight of fat in the sample}}{\text{Original weight of the sample}} \times 100$$

**3.2.5.2. Quality Parameters:****3.2.5.2.1. Estimation of total volatile basic nitrogen (TVB-N)**

TVB-N concentration was estimated by using the method given by Conway (1947). In brief, Conway cups and lids were washed and dried. Paraffin wax and vaseline in the ratio of 1:2 was melted and cooled. This was applied on the rims of cups. 1 ml of 0.02(N) H<sub>2</sub>SO<sub>4</sub> was added into the inner chamber of each cup. Lid was placed over the Conway cup covering the part of outer chamber and complete inner chamber. 1 ml of TCA extract was taken in the outer chamber followed by 1 ml of K<sub>2</sub>CO<sub>3</sub> solution. The unit was lidded and the contents were mixed by rotating the unit gently and then the unit was left overnight for reaction. The excess acid left in inner chamber was titrated against 0.02(N) NaOH using a drop of Toshiro's indicator. A reagent blank was done simultaneously. Total TVBN content was calculated with the formula given below:

$$1 \text{ ml of } 0.02(\text{N}) \text{ H}_2\text{SO}_4 = 0.28 \text{ mg of TVB-nitrogen}$$

**3.2.5.2.2. Estimation of Trimethyl-amine (TMA)**

TMA concentration was estimated by using the method given by Conway (1947). In brief, Conway cups and lids were washed and dried. Paraffin wax and vaseline in the ratio of 1:2 was melted and cooled. This was applied on the rims of cups. 1 ml of 0.02 H<sub>2</sub>SO<sub>4</sub> was added into the inner chamber of each cup. Lid was placed over the Conway cup covering the part of outer chamber and complete inner chamber. 1 ml of TCA extract was taken in the outer chamber followed by 0.5 ml of HCHO. The unit is rotated to ensure mixing and then 1 ml of K<sub>2</sub>CO<sub>3</sub> is added to outer chamber. The unit was lidded and the contents were mixed by rotating the unit gently and then the unit was left overnight for reaction. The excess acid left in inner chamber was titrated against 0.02(N) NaOH using a drop of Toshiro's indicator. A reagent blank was done simultaneously. Total TMA content was calculated with the formula given below:

$$1 \text{ ml of } 0.02 \text{ H}_2\text{SO}_4 = 0.28 \text{ mg of TMA nitrogen}$$

#### 3.2.5.2.3. Estimation of Peroxide value:

The PV of the lipid was determined from the lipid extract using iodometric method as described by Jacobs (1958). Ten gram of the sample was taken and ground well with 20 g anhydrous  $\text{Na}_2\text{SO}_4$ . The blend was shaken thoroughly in distilled chloroform for 5-10 min and filtered. Fat content in 10 ml aliquot was determined by evaporating it. In another 10 ml of aliquot, 20 ml of glacial acetic acid were poured and a pinch of potassium iodide was added. The flask was closed and sealed with a solution of KI. It was shaken well and kept in the dark place for 30 min. The flask was taken out and sides were washed with distilled water. A few drops of starch indicator were added and titrated immediately against N/500  $\text{Na}_2\text{S}_2\text{O}_3$ . The end was denoted by the disappearance of the blue colour. It is expressed as milliequivalent of  $\text{O}_2$  per kg of fat.

#### 3.2.5.2.4. Estimation of TBA:

TBARS estimation was done by using a scientific kit of make Hi-Media CCK023-1000.

#### 3.2.5.3. Microbiological characteristics:

Microbiological characteristics of fresh fish, brined fish and dry fish were carried out as per the Standard methods (APHA, 1984). Appropriate dilution of the homogenate was made in a physiological saline (0.85% Sodium Chloride solution made in water) plated, in duplicate, on nutrient agar, by spread plate method. The plates were incubated at  $37^\circ\text{C}$  for 48 hours and TPC was calculated accordingly. Like-wise mould count was enumerated on potato dextrose agar (PDA, 79) at  $30^\circ\text{C}$  for 5 days.

#### 3.2.5.4. Sensory analysis:

Determination of the quality of Dry and salted fish was made by trained panel of six (6) judges following 9-point hedonic scales (Peryan *et al.*, 1957). Comparison was carried out in terms of sensory characteristics such as odour, texture, and fish flavour intensity and general appearance. The panel was requested to rate each sensory feature of the salted product. The average score of 5 was considered to be the borderline of acceptability.

<i>9-point</i>	<i>Hedonic Scale</i>
9	Very much Extremely
8	Like Extremely
7	Like
6	Like Moderately
5	Like Slightly
4	Dislike Slightly
3	Dislike Moderately
2	Dislike
1	Extremely Dislike

#### 3.2.6. Statistical Analysis:

Statistical analysis of data was performed by using Microsoft Excel 2013. Results were presented as mean  $\pm$  standard deviation. Comparisons of mean values were determined by Duncan's Multiple Range Test. A probability level of 0.05 was used to find out the significance in all cases.

**Plate: 1. Dry fish collected from Jalda Khoti**



**Plate: 2. Dry fish collected from Junput Khoti**



**Plate: 3 and 4. Dry and Salted fish prepared in Laboratory condition and packed in packets (LDPE material)  
for storage studies**



**Plate: 5. Bamboo structure made for sun drying at FFSc**



**Plate: 6. Drying of Bombay duck in open sun**



**Plate: 7. Drying of Ribbon fish in open sun**



**Plate: 8. Drying of Ribbon fish in open sun**





*Chapter - 4*

**EXPERIMENTAL RESULTS**

#### 4.1. Dry fish production practices in Junput and Jalda khoti:

The assessment on different activities relating to fish trade particularly on dry fish was first taken up at Junput Khoti near Kontai, East Midnapore and the collected information from the fishermen involved in this industry are depicted in Table no 4 and 5.

**Table no. 4. Depicting the size of important fishes available in both the khotis.**

Name of the fish/species	Minimum size (Size in cm)	Maximum size (Size in cm)	Average size (Size in cm)
Ribbon fish	36	56	46
Bombay duck	20	26	23
<i>Setipinna phasa</i>	23	33	27.5

**Table no 5. Comparative study on the activities of two different khotis.**

Activities	Jalda Khoti	Junput Khoti
Fishing activities:		
Crafts use	Small mechanised boat (motorised)	Small mechanised boat (motorised)
Gear used	Bag net (Behundi jaal)	Bag net, trawl net
Duration of fishing	12 hrs (morning and evening)	12 hrs-24 hrs
Area of fishing	Within 3-5 kms from the shore line	Within 3-5 kms from the shore line
Drying activities:		
Species dried	Bombay duck ( <i>Harpodon nehereus</i> ), Ribbon fish	Ribbon fish ( <i>Lepturacanthus savala</i> ), Bombay duck

	( <i>Lepturacanthus savala</i> ), ( <i>Harpodon nehereus</i> ), <i>Setipinna phasa</i> , <i>Chanda nama</i> etc.	<i>Setipinna phasa</i> , Croakers ( <i>Johnius</i> spp) and small prawns and crabs.
<b>Fishes collected from</b>	Jalda and new Jalda landing centres.	Mainly Junput landing centre, occasionally also from Digha mohona (Shankarpur landing centre).
<b>Method of drying</b>	Open sun drying by either hanging using hooks (Bombay duck) or by spreading over the polythene on a raised platform (Ribbon fish, croakers, phasa, small crabs and prawns).	Open sun drying by either hanging using hooks (Bombay duck) or by spreading over the polythene on a raised platform (Ribbon fish, croakers, phasa, small crabs and prawns).
<b>Treatment prior to drying</b>	No treatments.	Dip treatment in brine and also using certain other chemical solution not revealed
<b>Period of treatment</b>	No treatment	Generally for one day, but may be extended upto 2-3 days depending on the weather condition.
<b>Period of drying</b>	5-6 days	3-4 days
<b>Season of khoti activities</b>	November to April	October to March
<b>Method of storage</b>	Packed in gunny bags and kept in dark place.	Packed in gunny bags and kept in dark place.

**Plates: 9-14 (Activities of Jalda Khoti)**

**9. Fish being harvested**



**10. Manually sorting of fish**



**11. Drying of ribbon fish using mat**



**12. Drying of Bombay duck fish**



**13. Drying of fish on a raised platform.**



**14. Packing of dry fish in gunny bags**



**Plates No. : 15-20 (Activities of Junput Khoti)**

**15. Harvesting of fish**



**16. Chemical treatments prior to drying**



**17. Salting of fishes before drying**



**18. Sun drying of Bombay duck fish**



**19. Sun drying of Ribbon fish**



**20. Fishes packed in gunny bags**



#### 4.2. Physical Characteristics of dry fishes (*Lepturacanthus savala* and *Harpodon nehereus*):

The total length of Bombay duck used for experiment ranged between (20-23) cm and ribbon fish ranged between (24-26) cm. The weight of the same species were varied from (4-7) g and (7-10) g respectively.

#### 4.3. Proximate composition and Quality parameters of dry fishes at Jalda Khoti:

Dry fish is the low cost dietary protein and are kept for longer period for future consumption. The purpose of this study is to determine proximate composition of two available marine dry fish, i.e. *Lepturacanthus savala* and *Harpodon nehereus*. The mean value of proximate composition for Bombay duck of Jalda Khoti was  $58.38 \pm 1.08$  %,  $18.22 \pm 1.14$ %,  $10.77 \pm 0.23$ %, and  $7.24 \pm 0.87$ % for protein, fat, ash and moisture respectively. The values for ribbon fishes were  $61.19 \pm 0.41$ %,  $15.36 \pm 0.85$ %,  $14.69 \pm 0.4$ %,  $8.08 \pm 1.2$ % for protein, fat, ash and moisture respectively.

**Table No. 6. Proximate composition of dry Bombay duck and Ribbon fish collected from Jalda Khoti**

Species	Protein	Fat	Ash	Moisture
<b>Bombay Duck</b>	$58.39 \pm 1.08$	$18.22 \pm 1.14$	$10.78 \pm 0.23$	$7.24 \pm 0.87$
<b>Ribbon Fish</b>	$61.19 \pm 0.41$	$15.37 \pm 0.86$	$14.69 \pm 0.4$	$8.08 \pm 1.17$

© Results are mean of three ( $n=3$ ) determinations with s.d.

The values obtained for quality parameters such as TVBN, TMA, PV, and TBA for collected dry Bombay duck were  $40.59 \pm 3.01$ %,  $27.73 \pm 0.39$ %,  $19.1 \pm 4.94$  mili-equi O<sub>2</sub>/kg and 3.61 mg MDA/ kg fat respectively. The values for ribbon fish for the same parameters in the given order were  $80.27 \pm 3.01$ %,  $40.32 \pm 1.25$ %,  $11.82 \pm 6.42$  mili equi O<sub>2</sub>/kg and 10.08mg MDA/ kg fat respectively. The mean values of total bacterial load and total fungal count for dry Bombay duck fish were  $1.17 \times 10^7$ cfu/g and  $1.08 \times 10^3$ cfu/g and for ribbon fish, the values were  $2.79 \times 10^7$ cfu/g and  $9 \times 10^2$ cfu/g respectively.

**Table No. 7. Biochemical quality parameters of dry Bombay duck and ribbon fish collected from Jalda Khoti**

Species	TMA (%)	TVBN (%)	PV (mili-equi O <sub>2</sub> )	TBA (mg MDA/ kg)
Bombay Duck	27.73 ± 0.39	40.59 ± 3.0	19.1 ± 4.94	3.61
Ribbon Fish	40.32 ± 1.25	80.27 ± 3.0	11.82 ± 6.42	10.08

© Results are mean of three (n=3) determinations with s.d

**Table No. 8. Microbial quality of dry Bombay duck and Ribbon fish collected from Jalda Khoti**

Species	TPC (log cfu/gm)	TFC (log cfu/gm)	Species	TPC (log cfu/gm)	TFC (log cfu/gm)
B1	7.100	2.954	R1	7.417	2.954
B2	7.033	3.100	R2	7.473	2.954
Average	7.067	3.027	Average	7.445	2.954

© B1 and B2 are the replicate of dry Bombay duck sample.

© R1 and R2 are the replicate of dry Ribbon fish sample.

#### 4.4. Proximate composition and Quality parameters of dry fish at Junput Khoti:

Dry fishes collected from Junput khoti are having different values for proximate composition than that of Jalda khoti. The mean values of proximate composition for protein, fat, ash and moisture dry Bombay duck were 57.08 ± 1.72%, 16.15 ± 1.22%, and 11.29 ± 0.19%, 11.81 ± 0.55% and for Ribbon fish, the values are 61.67 ± 1.39%, 12.97 ± 0.39%, 13.06 ± 0.72%, and 17.41 ± 1.39% respectively.

**Table No. 9. Proximate composition of dry Bombay duck and Ribbon fish collected from Junput Khoti**

Species	Protein	Fat	Ash	Moisture
Bombay Duck	57.08 ± 1.72	16.15 ± 1.22	11.29 ± 0.19	11.81 ± 0.55
Ribbon Fish	61.67 ± 1.39	12.97 ± 0.39	13.06 ± 0.72	17.41 ± 1.39

© Results are mean of three (n=3) determinations with s.d

The values for quality parameters such as TVB-N, TMA, PV, and TBA for collected dry Bombay duck at Junput khoti are  $26.56 \pm 2.36\%$ ,  $15.06 \pm 1.17\%$ ,  $5.24 \pm 0.21$  mili-equi  $O_2$ /kg, and  $4.52$ mg MDA/ kg fat respectively. The values for ribbon fish for the same parameters in the given order are  $65.89 \pm 4.69\%$ ,  $38.82 \pm 3.82\%$ ,  $34.72 \pm 2.99$  mili equi  $O_2$ /kg, and  $8.39$ mg MDA/ kg fat. The mean values of total bacterial load and total fungal count for dry Bombay duck fish were  $1.008 \times 10^6$ cfu/gm and  $9.9 \times 10^4$ cfu/gm respectively and for ribbon fish were  $1.55 \times 10^7$ cfu/gm and  $6 \times 10^4$  cfu/gm respectively.

**Table No. 10. Quality parameters of dry Bombay duck and ribbon fish collected from Junput Khoti**

Species	TMA (%)	TVBN (%)	PV(mili-equi $O_2$ )	TBA (mg MDA/ kg)
<b>Bombay Duck</b>	$15.06 \pm 1.17$	$26.56 \pm 2.35$	$5.24 \pm 0.21$	4.52
<b>Ribbon Fish</b>	$38.82 \pm 3.89$	$65.9 \pm 4.69$	$34.72 \pm 2.99$	8.39

© Results are mean of three (n=3) determinations with s.d.

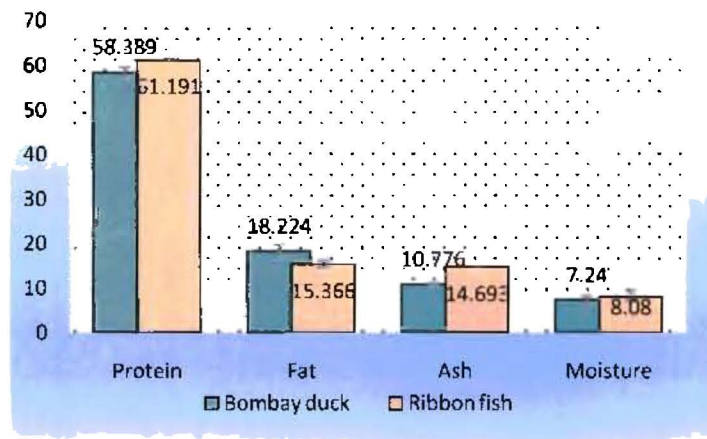
**Table No. 11. Microbial quality of dry Bombay duck and Ribbon fish collected from Junput Khoti**

Species	TPC (log cfu/ gm)	TFC (log cfu/ gm)	Species	TPC log cfu/gm	TFC log cfu/gm
<b>B1</b>	5.979	4.954	<b>R1</b>	7.015	4.687
<b>B2</b>	6.026	5.033	<b>R2</b>	7.316	4.857
<b>Average</b>	6.003	4.994	<b>Average</b>	7.165	4.772

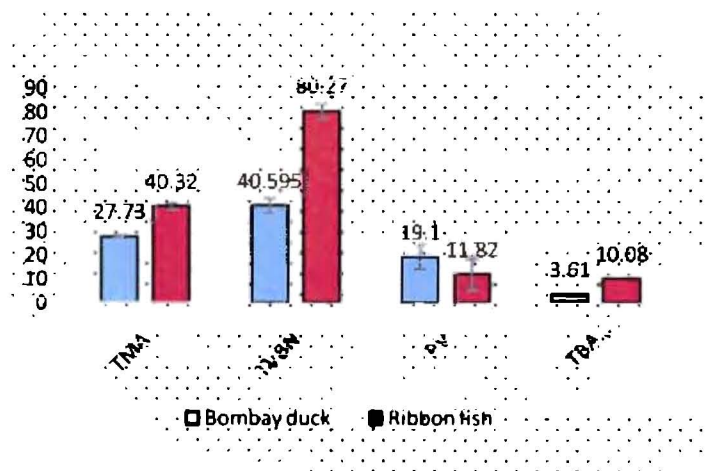
© B1 and B2 are the replicate of dry Bombay duck sample.

© R1 and R2 are the replicate of dry Ribbon fish sample.

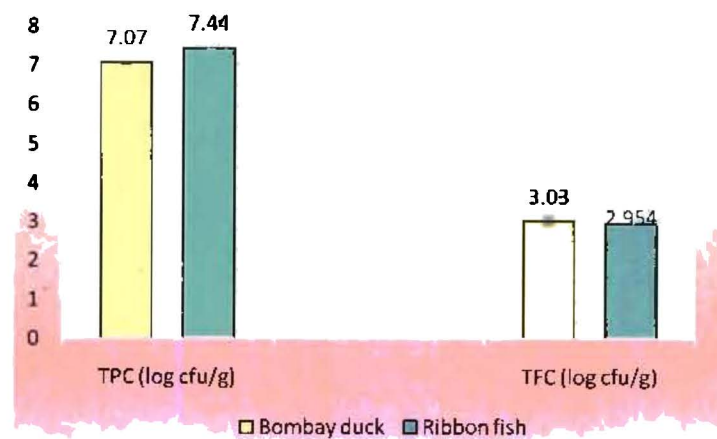
**Fig No. 2. Proximate composition of dry fishes at Jalda khoti**



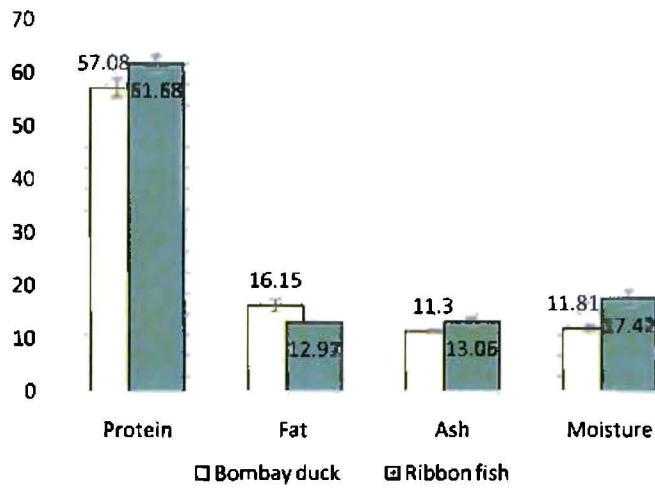
**Fig No.3. Biochemical quality parameters of dry fishes at Jalda khoti**



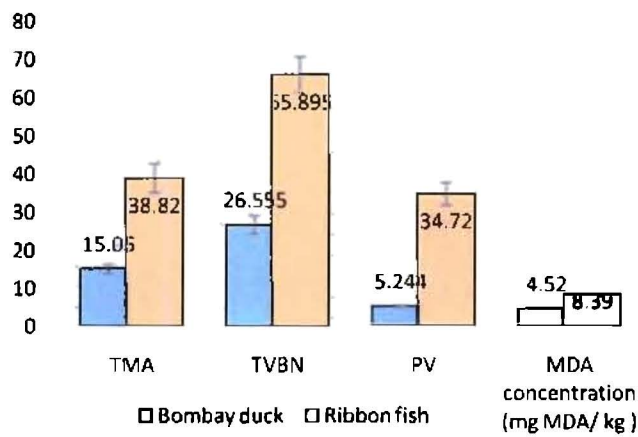
**Fig No. 4. Microbial characteristics of dry fishes at Jalda Khoti**



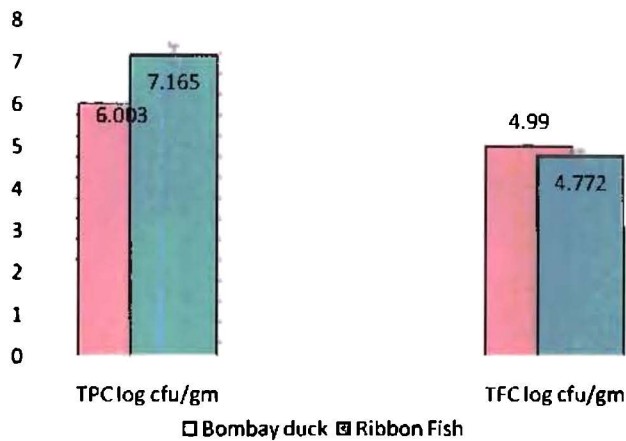
**Fig No. 5. Proximate composition of dry fishes at Junput Khoti**



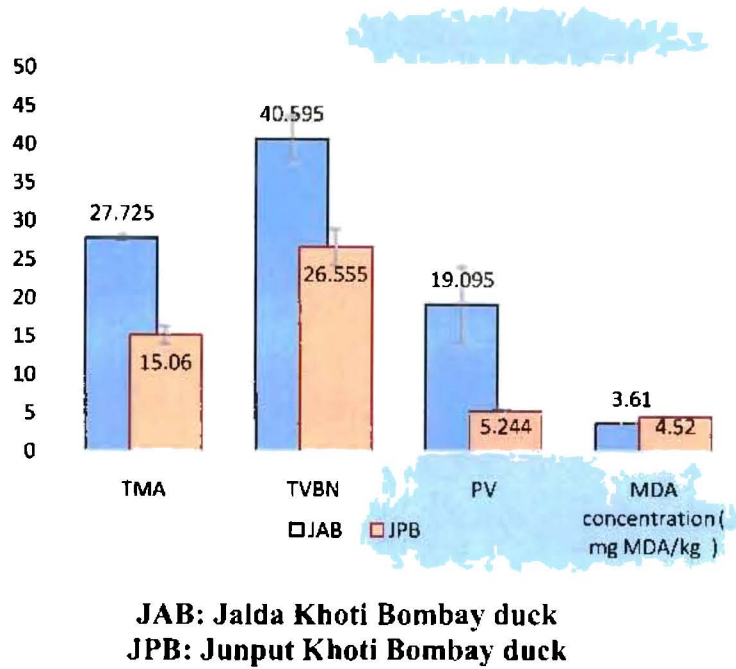
**Fig No. 6. Biochemical Quality parameters of dry fishes at Junput Khoti**



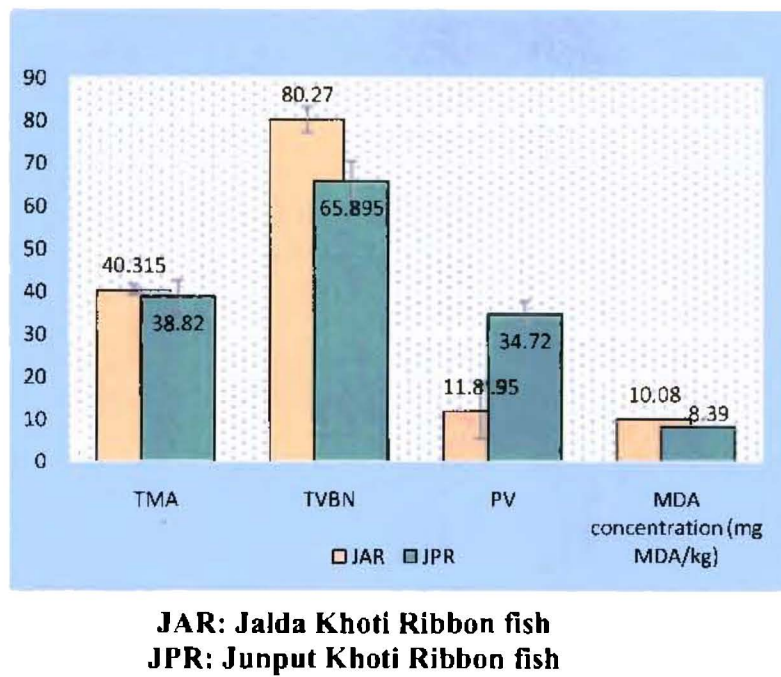
**Fig No. 7. Microbial characteristics of dry fishes at Junput Khoti**



**Fig no. 8.** Comparison between quality parameters of dry bombay duck collected from two khotis



**Fig no. 9.** Comparison between Biochemical quality parameters of dry Ribbon fish collected from two khotis



#### 4.5. Characteristics of fresh fishes:

Two commercially important marine species (i.e. *Harpodon nehereus* and *Lepturacanthus savala*) were considered for this experiment which is most commonly dried in these khoti areas. The fishes were collected in freshly iced condition and were analysed physically, biochemically and microbiologically to assess its degree of freshness. The fishes are treated with salt and different commercially available chemical preservatives and dried in open sun. The dry Bombay duck and ribbon fishes were investigated during 120 days storage at room temperature.

##### 4.5.1. Physical Characteristics:

The fishes were collected in fresh condition. The total length of *Harpodon nehereus* ranged between (20-27) cm with an average length of 23.5cm, and weight ranged from (33-107) grams with average weight of 60gms. The total length of *Lepturacanthus savala* ranged between (46-60) cm with an average length of 53cm. The weight ranged from (70-101) grams with an average weight of 85.5 grams.

##### 4.5.2. Proximate Composition:

The proximate composition of freshly collected *Harpodon nehereus* and *Lepturacanthus savala* were analysed to determine its nutritional quality before drying. The mean values of protein, fat, ash and moisture of Bombay duck were found 8.03±0.09 %, 2.01±0.09%, 2.59±0.27%, 86.72±0.52 % (Table. 12) and for ribbon fish 17.39 ± 0.68 %, 3.92 ± 1.22%, 3.31 ± 0.4%, 64.19 ± 0.73% (Table.12) respectively.

**Table No. 12. Proximate composition of Fresh Bombay Duck and Ribbon fish.**

Species	Protein	Fat	Ash	Moisture
Bombay Duck	8.03 ± 0.09	2.01 ± 0.09	2.59 ± 0.27	86.72 ± 0.52
Ribbon Fish	17.39 ± 0.68	3.915 ± 1.22	3.31 ± 0.4	64.19± 0.73

© Results are mean of three (n=3) determinations with s.d

##### 4.5.3. Biochemical parameters:

The biochemical parameters of fresh *Harpodon nehereus* and *Lepturacanthus savala* such as TVBN, TMA, PV,  $a_w$  were analysed to assess its degree of freshness. In case fresh Bombay duck the mean values TVBN, TMA, PV, TBA were 15.31 ± 2.41 mg%, 3.79 ± 0.55 mg%, 1.69 ± 2.39 mili equi O<sub>2</sub>/kg, and 0.00 respectively. The

mean values for fresh ribbon fish were  $13.08 \pm 0.26$  mg%, 0,  $10.51 \pm 0.64$  mili equi  $O_2$ /kg and 0 in the same order respectively.

**Table no. 13. Biochemical quality parameters of fresh Bombay duck and Ribbon fish.**

Species	TMA (mg %)	TVBN (mg %)	PV (mili-equi $O_2$ )	TBA (mg MDA/kg fat)
Bombay Duck	$3.79 \pm 0.55$	$15.31 \pm 2.4$	$1.69 \pm 2.39$	0
Ribbon Fish	0	$13.08 \pm 0.26$	$10.51 \pm 0.64$	0

© Results are mean of three ( $n=3$ ) determinations with s.d.

#### 4.5.4. Microbiological characteristics:

The mean values of total bacterial load (TPC) and total fungal count (TFC) for fresh Bombay duck fish were  $2.18 \times 10^4$  cfu/gm and 0 and in Ribbon fish, the values were  $2.5 \times 10^4$  cfu/gm and 0 respectively.

**Table no. 14. Microbial quality of fresh Bombay duck and Ribbon fish.**

	Bombay Duck	Ribbon fish
TPC (cfu/gm)	$2.18 \times 10^4$	$2.485 \times 10^4$
TPC (log cfu/gm)	4.338	4.395
TFC (cfu/gm)	0	0
TFC (log cfu/gm)	0	0

#### 4.6. Drying studies of *Harpodon nehereus* and *Lepturacanthus savala*:

Drying was done in open sun after different treatments (i.e. Dip treatment in saturated brine (Control), smearing with dry salt (NaCl) and 0.25% potassium sorbate ( $T_1$ ) and dip treatment in saturated brine and 0.3% sodium benzoate  $T_2$ ). During the period of drying, the temperature varied between  $31.6^\circ C$  to  $39.7^\circ C$  with an average atmospheric temperature of  $35.1^\circ C \pm 4.95$ . The relative humidity during drying period was varied from 48% to 72%. The period of drying was taken for almost 9-10 days to reduce the moisture level below 15% to 20%. Level moisture was observed at frequent intervals to assess the rate of drying. The change of moisture content at frequent intervals during drying period is expressed below:

**Table no. 15.** Moisture content at frequent intervals during open sun drying

days	Saturated brine (Control)		Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )		Saturated brine and 0.3% Sodium benzoate (T <sub>2</sub> )	
	<i>H. nehereus</i>	<i>L. savala</i>	<i>H. nehereus</i>	<i>L. savala</i>	<i>H. nehereus</i>	<i>L. savala</i>
	0	86.72±0.1 %	64.19±0.3%	86.72±0.1%	64.19± 0.2%	86.72± 0.4%
3 <sup>rd</sup>	63.41±0.1 %	47.19±0.2%	56.17±0.7%	41.91±0.9%	64.14±0.7 %	39.61±0.2 %
5 <sup>th</sup>	49.60± 0.3%	21.58±0.8%	35.79±0.4%	25.34± 0.1%	47.42±0.4 %	19.89± 0.3%
7 <sup>th</sup>	22.59±0.6%	19.33±0.4%	25.14±0.7%	12.23± 0.6%	32.32± 0.4%	19.12± 0.8%
10 <sup>th</sup>	6.91±0.71%	8.11± 0.5%	7.42 ± 0.8%	8.82 ± 0.1%	6.9 ±0.33%	17.59±0.4 %

\*Results are mean of three (n=3) determinations with sd.

# Values of moisture content vary significantly ( $p < 0.05$ ) within drying period and insignificantly ( $p > 0.05$ ) between the treatment for a particular species.

#### 4.7. Nutritional qualities of dry fishes:

##### 4.7.1. Analysis of Proximate composition:

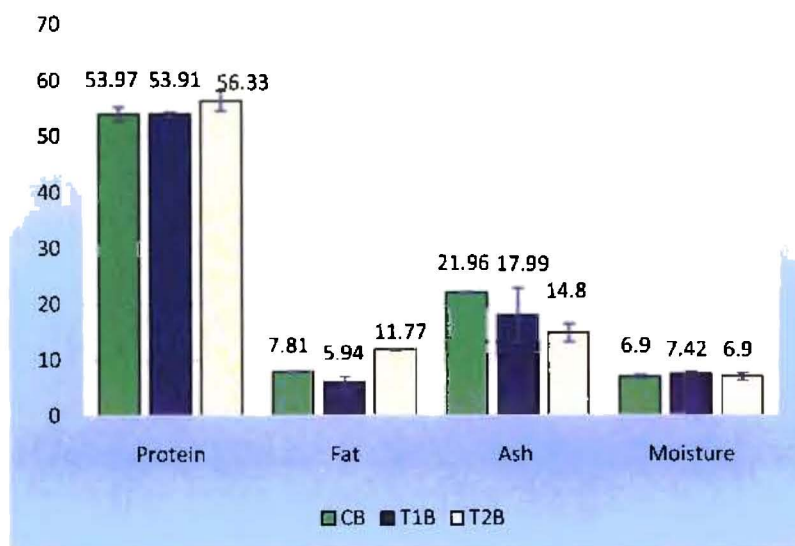
After drying, the values for protein, fat, ash and moisture of dry Bombay duck for three different treatments were found in a range of 53.91 to 56.33%, 5.94 to 11.77%, 14.78 to 21.96 % and 6.9 to 7.42% respectively. Similarly the values for protein, fat, ash and moisture of dry Ribbon fish for different treatments were found in a range of 52.33 to 53.76 %, 11.53 to 13.79%, 17.86 to 23.0 % and 8.82 to 17.59%.

**Table No. 16.** Proximate Composition of Dry Bombay Duck and Ribbon Fish

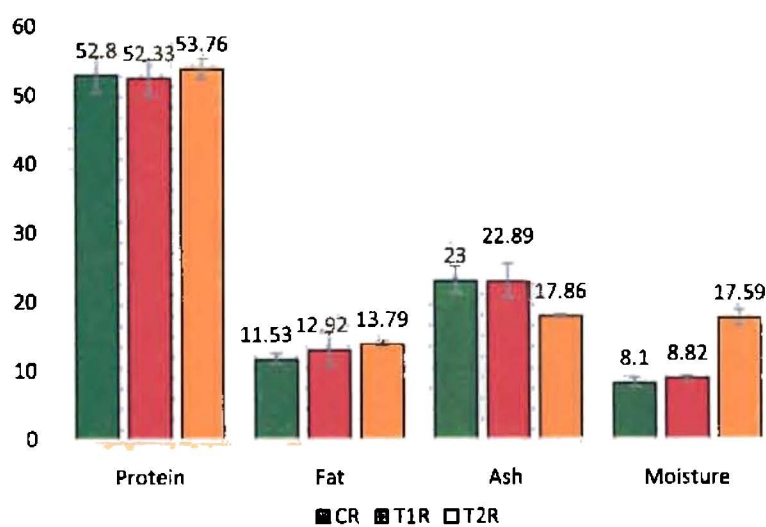
Species	Parameters	Saturated brine (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
<i>H. nehereus</i>	Protein (%)	53.97 ± 1.23	53.91 ±0.35	56.33 ± 1.8
	Fat (%)	7.81 ± 0.09	5.94 ±1.02	11.77 ± 0.23
	Ash (%)	21.96 ± 0.08	17.99± 4.75	14.78 ± 1.61
	Moisture (%)	6.90 ± 0.3	7.42 ±0.44	6.9 ± 0.66
<i>L. savala</i>	Protein (%)	52.8± 2.6	52.33 ± 2.56	53.76 ± 1.45
	Fat (%)	11.53 ± 0.78	12.92 ± 2.45	13.79 ± 0.42
	Ash (%)	23.0 ± 1.99	22.89 ± 2.53	17.86± 0.21
	Moisture (%)	8.11 ± 0.74	8.82± 0.27	17.59±1.17

\*Results are mean of three (n=3) determinations with s.d.

**Fig No.12. Proximate composition of dry Bombay duck of different treatments**



**Fig No.13. Proximate composition of dry ribbon fish of different treatment**



#### 4.8. Storage Study of the dry fishes:

After drying, the fishes are packed in polythene packets and kept in ambient temperature at room, for its further storage study. The storage study of the dry fishes of different treatments is done at monthly intervals for a period of four months. During storage study the parameters which are observed are describing below:

##### 4.8.1. Biochemical quality assessment:

###### 4.8.1.1. Total Volatile Base Nitrogen (TVBN):

The TVBN values of dry Bombay duck shows an increase trend in all samples of different treatments of its storage period. The TVBN content of T<sub>1</sub> samples had increased from 26.09 mg% to 48.87 mg%, during its four months storage period. In case of controlled sample, TVBN content of dry Bombay duck had increased from 24.79 mg% to 41.18 mg% and for Bombay duck of T<sub>2</sub> sample, the value increased from 19.67 mg% to 29.94 mg% during storage periods. Similarly, the TVBN values for ribbon fishes of T<sub>1</sub> sample and the controlled sample had increased from 13.25 mg % to 42.52 mg% and 12.28 mg% to 49.53 mg% respectively. However, the value for ribbon fishes of T<sub>2</sub> sample had increased from 12.84 mg% to 36.49 mg% during storage study.

###### 4.8.1.2. Tri-methylamine (TMA):

The TMA content for dry Bombay duck of control sample (C) and T<sub>1</sub> sample were increased from 11.89 mg% to 19.53 mg% and 10.4 mg% to 24.82 mg% respectively. Whereas the increasing trend of TMA content was less in dry Bombay duck of T<sub>2</sub> sample from 11.03 mg% to 15.24 mg% during storage study. Similarly, the TMA values for ribbon fishes of C, T<sub>1</sub> and T<sub>2</sub> samples were increased from 5.37 mg% to 23.11 mg%, from 0 mg% to 23.11% and from 0 mg% to 16.59 mg% respectively, during its period of storage.

###### 4.8.1.3. Peroxide value (PV):

In case of peroxide value, increasing trend was obtained during 120 days of storage study of both the dry Bombay duck and Ribbon fish. The peroxide value as mili-equivalent O<sub>2</sub>/kg fat for dry Bombay duck of controlled sample (C), potassium T<sub>1</sub> sample and T<sub>2</sub> sample were increased from 12.22 to 32.14, 6.88 to 35.32 and 9.68

to 16.98 respectively during storage study. Similarly, in case of dry ribbon fish of three respective samples, the peroxide value increased from 16.26 to 51.22, 8.46 to 51.07 and 17.91 to 30.05, during 120 days storage period.

#### 4.8.1.4. Thio-barbituric acid (TBA):

TBA value measures the degree of rancidity in a form of malano-aldehyde in mg produced in fish muscle. During storage, the TBA value as mg MDA/ kg fish for dry Bombay duck of controlled sample (C), T<sub>1</sub> sample and T<sub>2</sub> sample were increased from 2.84 to 3.79, 3.82 to 7.36 and 3.14 to 6.27 respectively. At the same time, the value increased for dry ribbon fish of C sample, T<sub>1</sub> sample and T<sub>2</sub> sample were from 3.31 to 7.33, 2.99 to 7.3 and 3.93 to 8.22 respectively.

**Table No. 17. Changes of TVBN (mg %) value in dry Bombay duck during storage period.**

days	Saturated brine (Control)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	24.79 ± 2.3 <sup>ab</sup>	26.09 ± 2.3 <sup>abc</sup>	19.67 ± 0.96 <sup>a</sup>
30 <sup>th</sup>	30.53 ± 2.9 <sup>bcd</sup>	28.09 ± 2.2 <sup>abcd</sup>	24.77 ± 1.55 <sup>ab</sup>
60 <sup>th</sup>	35.91 ± 2.1 <sup>cde</sup>	32.6 ± 5.0 <sup>bcdc</sup>	27.89 ± 2.0 <sup>abcd</sup>
90 <sup>th</sup>	37.18 ± 4.8 <sup>de</sup>	36.46 ± 4.7 <sup>de</sup>	29.72 ± 4.67 <sup>bcd</sup>
120 <sup>th</sup>	41.18 ± 2.4 <sup>ef</sup>	48.87 ± 5.6 <sup>f</sup>	29.94 ± 4.70 <sup>bcd</sup>

© \*Results are mean of three (n=3) determinations with s.d.

# Values of TVBN vary significantly ( $p < 0.05$ ) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row ( $p < 0.05$ ).

**Table No. 18. Changes of TVBN (mg %) value in dry Ribbon fish during storage**

days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	12.28 ± 1.6 <sup>a</sup>	13.25 ± 0.7 <sup>a</sup>	12.84 ± 0.17 <sup>a</sup>
30 <sup>th</sup>	39.37 ± 2.4 <sup>de</sup>	14.97 ± 1.9 <sup>abc</sup>	13.87 ± 0.21 <sup>ab</sup>
60 <sup>th</sup>	37.6 ± 1.83 <sup>de</sup>	23.24 ± 4.1 <sup>abcd</sup>	33.58 ± 5.26 <sup>bcdc</sup>
90 <sup>th</sup>	44.07 ± 3.17 <sup>e</sup>	41.24 ± 2.1 <sup>de</sup>	34.31 ± 9.71 <sup>cde</sup>
120 <sup>th</sup>	49.53 ± 4.7 <sup>e</sup>	42.52 ± 4.6 <sup>de</sup>	36.49 ± 4.69 <sup>de</sup>

© Results are mean of three (n=3) determinations with s.d.

# Values of TVBN vary significantly ( $p < 0.05$ ) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row ( $p < 0.05$ ).

**Table No. 19. Changes of TMA (mg %) value in dry Bombay duck during storage period.**

days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	11.89 ± 1.87	10.4 ± 4.9	11.03 ± 3.58
30 <sup>th</sup>	14.12 ± 0.6	9.33 ± 1.31	11.26 ± 1.30
60 <sup>th</sup>	15.28 ± 1.81	21.81 ± 6.51	12.53 ± 3.74
90 <sup>th</sup>	19.77 ± 8.12	24.82 ± 2.4	14.88 ± 2.37
120 <sup>th</sup>	19.53 ± 5.12	24.82 ± 3.72	15.24 ± 2.73

© Results are mean of three (n=3) determinations with s.d.

# Values of TMA content vary significantly ( $p < 0.05$ ) within storage period and insignificantly ( $p > 0.05$ ) between the treatments.

**Table No. 20.** Changes of TMA (mg %) value in dry Ribbon fish during storage

days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine and 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	5.37 ± 1.9 <sup>ab</sup>	0 <sup>a</sup>	0 <sup>a</sup>
30 <sup>th</sup>	14.08 ± 0.17 <sup>cd</sup>	7.0 ± 0.3 <sup>abc</sup>	7.04 ± 0.25 <sup>abc</sup>
60 <sup>th</sup>	17.20 ± 3.17 <sup>dc</sup>	16.32 ± 4.62 <sup>dc</sup>	10.83 ± 2.27 <sup>bcd</sup>
90 <sup>th</sup>	17.4 ± 4.92 <sup>dc</sup>	17.42 ± 1.45 <sup>dc</sup>	15.1 ± 1.94 <sup>dc</sup>
120 <sup>th</sup>	23.11 ± 4.67 <sup>c</sup>	23.11 ± 4.67 <sup>c</sup>	16.59 ± 4.69 <sup>dc</sup>

© Results are mean of three (n=3) determinations with s.d

# Values of TMA vary significantly (p < 0.05) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row (p < 0.05).

**Table No. 21.** Changes of peroxide value (mili-equi O<sub>2</sub> / kg fat) in dry Bombay duck during storage period

days	Saturated brine (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine and 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	12.22 ± 1.57 <sup>ab</sup>	6.88 ± 2.17 <sup>a</sup>	9.68 ± 1.1 <sup>a</sup>
30 <sup>th</sup>	15.45 ± 2 <sup>ab</sup>	13.37 ± 2.6 <sup>ab</sup>	10.71 ± 3.37 <sup>ab</sup>
60 <sup>th</sup>	15.62 ± 3.2 <sup>abc</sup>	23.64 ± 3.15 <sup>bcd</sup>	15.03 ± 3.47 <sup>ab</sup>
90 <sup>th</sup>	19.2 ± 2.01 <sup>abcd</sup>	29.14 ± 1.86 <sup>cd</sup>	17.21 ± 1.38 <sup>abc</sup>
120 <sup>th</sup>	32.14 ± 5.05 <sup>d</sup>	35.32 ± 0.09 <sup>d</sup>	16.98 ± 1.56 <sup>abc</sup>

© Results are mean of three (n=3) determinations with s.d

# Values of PV vary significantly (p < 0.05) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row (p < 0.05).

**Table No. 22. Changes of peroxide value (mili-equi O<sub>2</sub> / kg fat) in dry Ribbon fish during storage period.**

days	Saturated brine (Control) (C)		Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )		Saturated brine % 0.3% Sodium benzoate (T <sub>2</sub> )	
	μM MDA	mg MDA/kg	μM MDA	mg MDA/kg	μM MDA	mg MDA/kg
0 <sup>th</sup>	16.26 ± 3.22		8.46 ± 1.39		17.91 ± 0.22	
30 <sup>th</sup>	30.94 ± 4.75		16.84 ± 2.64		21.75 ± 4.01	
60 <sup>th</sup>	31.23 ± 1.98		29.08 ± 3.98		38.31 ± 4.17	
90 <sup>th</sup>	24.83 ± 1.51		21.16 ± 2.25		24.37 ± 1.46	
120 <sup>th</sup>	51.22 ± 3.15		51.07 ± 7.83		30.05 ± 3.87	

©Results are mean of six (n=6) determinations with s.d.

# Values of PV content vary significantly ( $p < 0.05$ ) within storage period and insignificantly ( $p > 0.05$ ) between the treatments.

**Table No. 23. Changes of TBA value (mg MDA / kg fish) in dry Bombay duck during storage period.**

days	Saturated brine (Control) (C)		Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )		Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )	
	μM MDA	mg MDA/kg	μM MDA	mg MDA/kg	μM MDA	mg MDA/kg
0 <sup>th</sup>	3.942±0.2	2.84±0.2 <sup>a</sup>	5.295±0.1	3.82±0.1 <sup>ab</sup>	4.35±0.4	3.14±0.2 <sup>a</sup>
30 <sup>th</sup>	4.11±0.21	2.96±0.21 <sup>a</sup>	6.56±0.15	4.73±0.15 <sup>abc</sup>	4.93±0.35	3.55±0.35 <sup>ab</sup>
60 <sup>th</sup>	3.49±0.11	2.52±0.11 <sup>a</sup>	7.43±0.27	5.35±0.27 <sup>abc</sup>	5.38±0.17	3.87±0.17 <sup>ab</sup>
90 <sup>th</sup>	5.42±0.2	3.9±0.2 <sup>ab</sup>	5.58±0.49	4.02±0.49 <sup>ab</sup>	5.13±0.24	3.7±0.24 <sup>ab</sup>
120 <sup>th</sup>	5.25±0.22	3.79±0.22 <sup>ab</sup>	10.21±0.15	7.36±0.15 <sup>c</sup>	8.70±0.36	6.27±0.36 <sup>bc</sup>

©Results are mean of three (n=3) determinations with s.d.

# Values of TBA vary significantly ( $p < 0.05$ ) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row ( $p < 0.05$ ).

**Table No. 24. Changes of TBA value (mg MDA / kg fish) in dry Ribbon fish during storage period.**

days	Saturated brine (Control) (C)		Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )		Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )	
	µM MDA	mg MDA/kg	µM MDA	mg MDA/kg	µM MDA	mg MDA/kg
0 <sup>th</sup>	4.60±0.12	3.31±0.12	4.15±0.1	2.99±0.1	5.46±2.1	3.93±2.1
30 <sup>th</sup>	4.76±1.4	3.43±1.4	5.70±2.2	4.11±2.2	5.46±0.33	3.93±0.33
60 <sup>th</sup>	5.34±0.09	3.85±0.09	7.43±3.1	5.35±3.1	7.67±1.1	5.53±1.1
90 <sup>th</sup>	5.05±2.4	3.64±2.4	6.28±1.9	4.52±1.9	5.74±0.13	4.14±0.13
120 <sup>th</sup>	10.17±0.2	7.33±0.2	10.13±2.1	7.30±2.1	11.40±1.7	8.22±1.7

© Results are mean of three (n=3) determinations with s.d.

# Values of TBA content vary significantly ( $p < 0.05$ ) within storage period and insignificantly ( $p > 0.05$ ) between the treatments.

#### 4.8.2. Microbiological quality assessment:

##### 4.8.2.1. Total Plate Count:

The total plate count of both dry fishes (i.e. Bombay duck and Ribbon fish) of different treatments showed an increasing trend during storage study. The values of TPC for dry Bombay duck as cfu/gm for controlled sample (C), T<sub>1</sub> sample and T<sub>2</sub> sample were reached upto  $4.365 \times 10^8$ ,  $2.52 \times 10^7$  and  $4.05 \times 10^7$  at the end of 120 days storage period. Similarly, for dry ribbon fish, the values increased upto a tune of  $1.034 \times 10^9$ ,  $1.98 \times 10^7$  and  $3.24 \times 10^7$  for Bombay duck and Ribbon fish of three different treatments.

##### 4.8.2.2. Total Fungal Count:

Total Fungal count of dry Bombay duck and ribbon fish was measured at one month interval for a period of 120 days. At the end of the storage period, the total fungal count for dry Bombay duck as cfu/gm for C, T<sub>1</sub> and T<sub>2</sub> samples were observed  $5.4 \times 10^6$ ,  $3.69 \times 10^6$  and  $2.7 \times 10^6$  respectively, whereas for dry ribbon fish, the value increased upto  $9.6 \times 10^7$ ,  $6.3 \times 10^6$  and  $8.1 \times 10^6$  for the same respective treatments.

**Table No. 25. Changes of Total Plate Counts of dry Bombay duck during storage study**

days	Saturated brine (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	4.739±0.08 <sup>a</sup>	4.377±0.17 <sup>a</sup>	4.474±0.1 <sup>a</sup>
30 <sup>th</sup>	5.605±0.7 <sup>abc</sup>	5.285±0.14 <sup>ab</sup>	5.265±0.6 <sup>ab</sup>
60 <sup>th</sup>	6.69±0.05 <sup>cdef</sup>	6.115±0.08 <sup>bcd</sup>	6.165±0.3 <sup>bcd</sup>
90 <sup>th</sup>	7.78± 0.1 <sup>fg</sup>	6.41±0.03 <sup>bcd</sup>	5.707±0.2 <sup>abc</sup>
120 <sup>th</sup>	8.626±0.01 <sup>g</sup>	7.266±0.2 <sup>dclg</sup>	7.55±0.07 <sup>cig</sup>

©Results are mean of six (n=6) determinations with s.d.

# Values of TPC vary significantly ( $p < 0.05$ ) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row ( $p < 0.05$ ).

**Table No.26. Change of Total Plate Counts of dry Ribbon fish during storage study.**

days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	5.594±0.08 <sup>abc</sup>	4.693±0.2 <sup>a</sup>	5.048±0.07 <sup>ab</sup>
30 <sup>th</sup>	5.574±0.2 <sup>abc</sup>	5.011±0.08 <sup>ab</sup>	5.468±0.36 <sup>abc</sup>
60 <sup>th</sup>	6.584±0.03 <sup>cd</sup>	5.693±0.06 <sup>abc</sup>	6.032±0.05 <sup>abcd</sup>
90 <sup>th</sup>	6.635±0.02 <sup>cd</sup>	6.158±0.06 <sup>bcd</sup>	6.266±0.01 <sup>bcd</sup>
120 <sup>th</sup>	8.855±0.2 <sup>e</sup>	7.24±0.08 <sup>d</sup>	7.444±0.09 <sup>de</sup>

©Results are mean of six (n=6) determinations with s.d.

# Values of TPC vary significantly ( $p < 0.05$ ) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row ( $p < 0.05$ ).

**Table No. 27. Changes of Total Fungal Counts of dry Bombay duck during storage study.**

days	Saturated brine (Control)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	4.097±0.05 <sup>abc</sup>	3.732±0.4 <sup>ab</sup>	3.125±0.18 <sup>a</sup>
30 <sup>th</sup>	4.454±0.7 <sup>abc</sup>	4.105±0.74 <sup>abc</sup>	3.975±0.03 <sup>abc</sup>
60 <sup>th</sup>	4.758±0.28 <sup>bcd</sup>	4.626±0.52 <sup>abc</sup>	3.454±1.75 <sup>ab</sup>
90 <sup>th</sup>	6.604±0.08 <sup>c</sup>	5.434±0.14 <sup>cdc</sup>	5.354±0.11 <sup>cdc</sup>
120 <sup>th</sup>	6.644±0.12 <sup>c</sup>	6.562±0.007 <sup>c</sup>	6.193±0.34 <sup>de</sup>

©Results are mean of three (n=3) determinations with s.d.

# Values of TFC vary significantly ( $p < 0.05$ ) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row ( $p < 0.05$ ).

**Table No. 28. Changes of Total Fungal Counts of dry Ribbon fish during storage study**

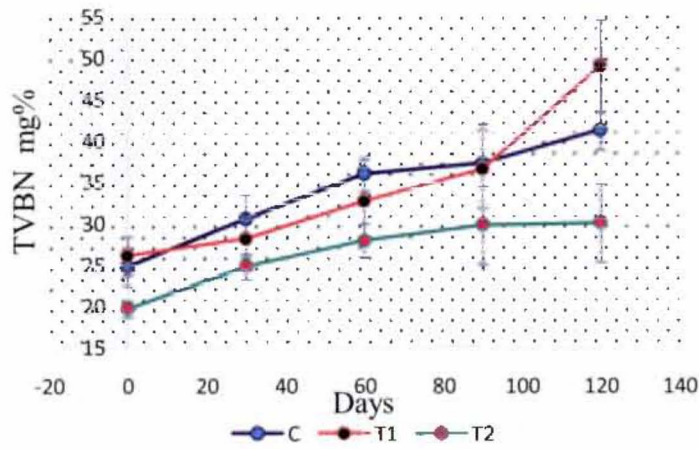
Days	Saturated brine (Control) (C)	Dry salt and 0.25% potassium sorbate (T <sub>1</sub> )	Saturated brine & 0.3% Sodium benzoate (T <sub>2</sub> )
0 <sup>th</sup>	0	0	0
30 <sup>th</sup>	3.012±0.07 <sup>bc</sup>	0	1.201±0.08 <sup>ab</sup>
60 <sup>th</sup>	4.193±0.3 <sup>cd</sup>	2.862±0.13 <sup>bc</sup>	2.954±0 <sup>bc</sup>
90 <sup>th</sup>	6.23±0.16 <sup>ef</sup>	5.477±0.02 <sup>de</sup>	5.105±0.2 <sup>de</sup>
120 <sup>th</sup>	7.96±0.03 <sup>f</sup>	6.622±0.25 <sup>ef</sup>	6.408±0.71 <sup>ef</sup>

©Results are mean of three (n=3) determinations with s.d.

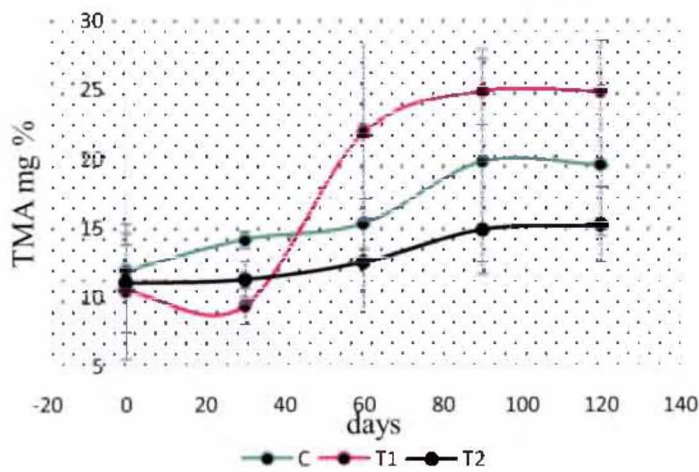
# Values of TFC vary significantly ( $p < 0.05$ ) between the treatment and storage period.

@ Values with different superscript letter are significantly different within a row ( $p < 0.05$ ).

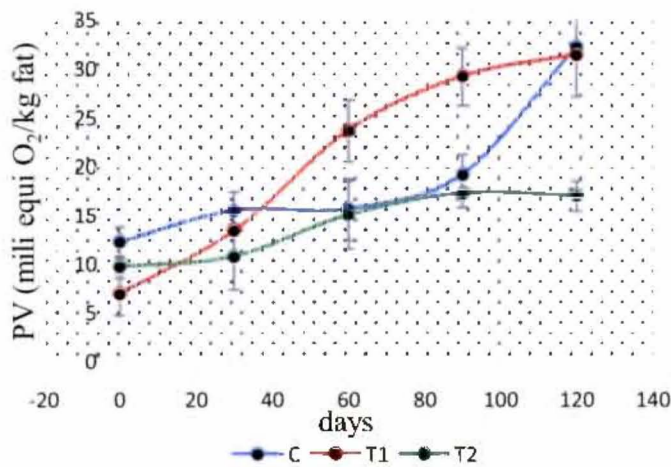
**Fig No.14.** Changes of TVBN value in dry Bombay duck during storage



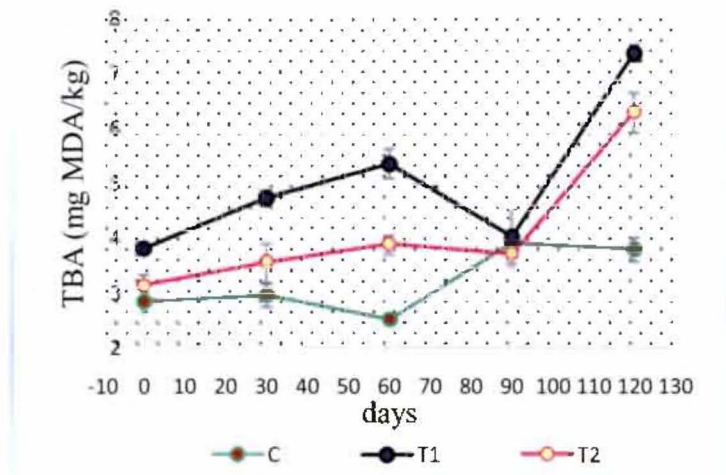
**Fig No. 15.** Changes of TMA value in dry Bombay duck during storage



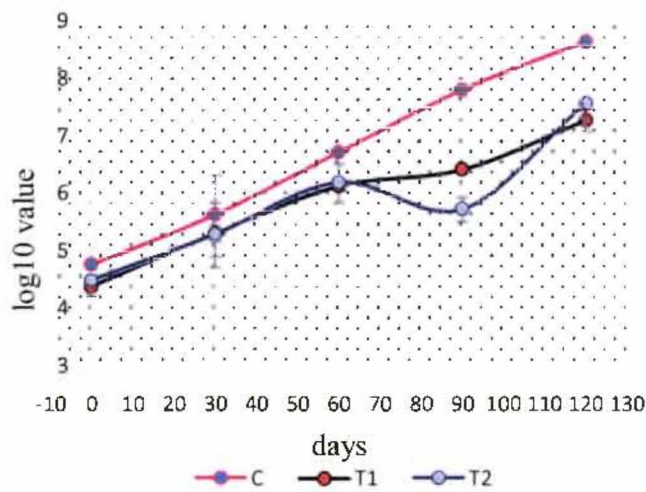
**Fig No.16.** Changes of PV in dry Bombay duck during storage



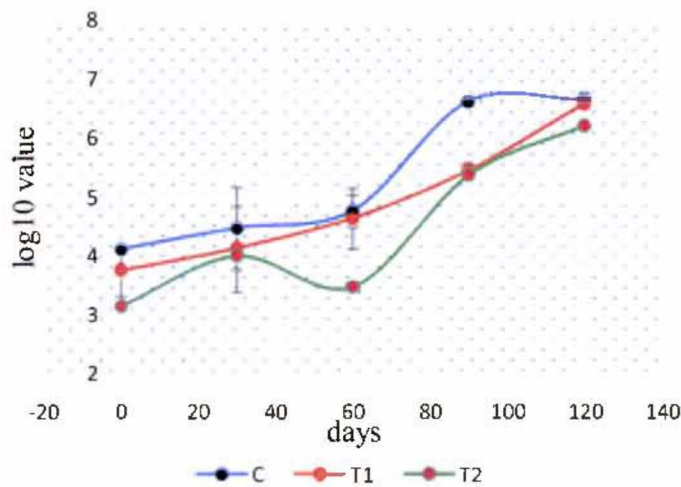
**Fig No.17.** Changes of TBA values in dry Bombay duck during storage



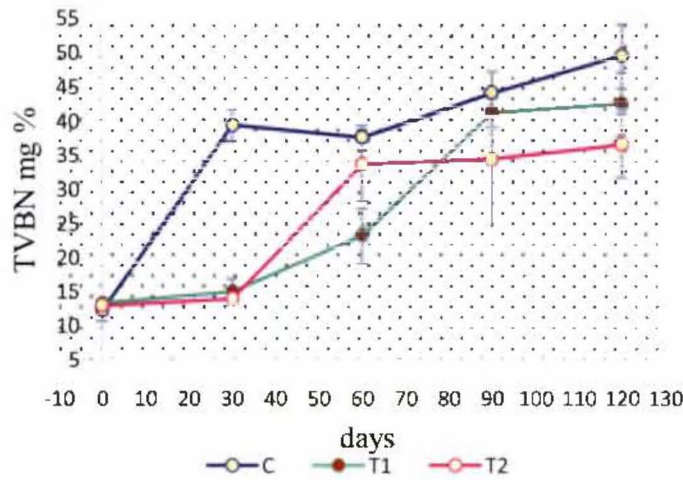
**Fig No.18.** Changes of TPC log value in dry Bombay duck during storage



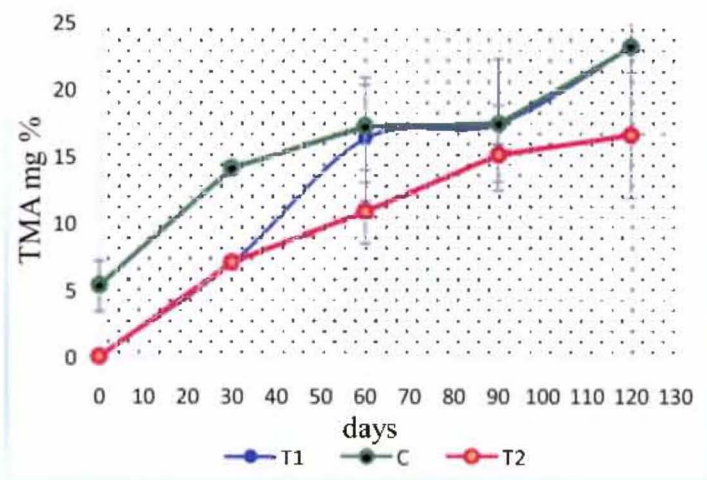
**Fig No.19.** Changes of TFC log value in dry Bombay duck during storage



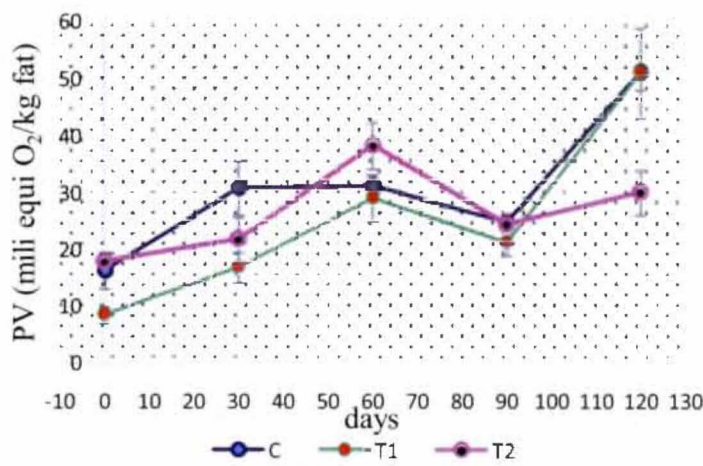
**Fig No.20.** Changes of TVBN value in dry Ribbon fishes during storage



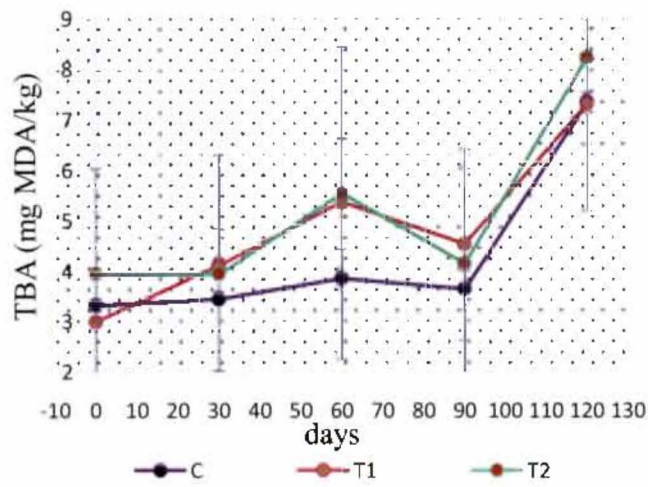
**Fig No.21.** Changes of TMA value in dry Ribbon fishes during storage



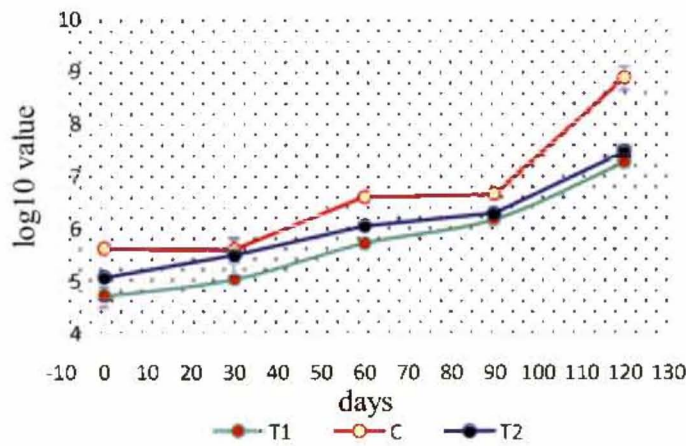
**Fig No.22.** Changes of PV in dry Ribbon fishes during storage



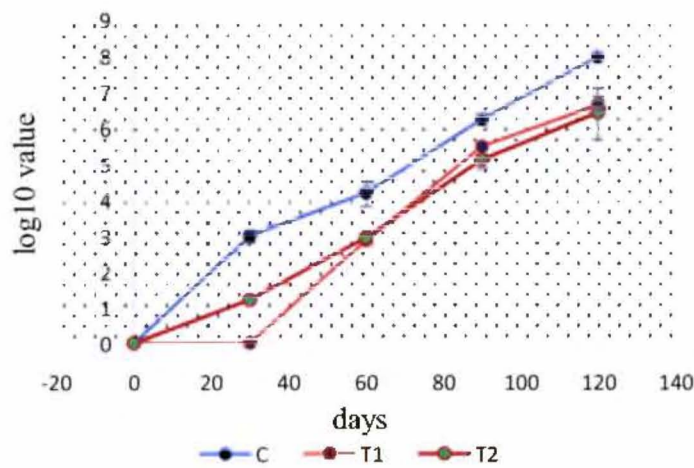
**Fig No.23.** Changes of TBA values in dry ribbon fish during storage



**Fig No.24.** Changes of TPC log value in dry Ribbon fishes during storage



**Fig No. 25.** Changes of log TFC values in dry ribbon fishes during storage



#### 4.9. Sensory Evaluation:

The sensory evaluation was done for both samples of different treatments based on 9 point Hedonic scale by expertise panellists. The results shows that the overall acceptability of both dry Bombay duck and ribbon fishes of C samples were comparatively poor than the treated samples during 120 days storage period (Table. 29-31). The colour of T<sub>1</sub> and T<sub>2</sub> samples were become light brownish after a period of 90 days storage but the odour and texture were still good at the end of the storage. The texture of C samples became fragile in nature during end of the storage period which reduces its overall acceptability.

**Table No. 29. Changes of organoleptic qualities of Control (C) samples during storage study**

Days	Species with treatments	Colour and appearance	Odour	Texture	Fish Flavour Intensity	Overall acceptability
0th	CB	7.67±0.52	8.33±0.52	8.00±0.00	9.00±0.0	8.25 ± 0.49
	CR	8.00±0.00	9.00±0.00	8.33±0.57	8.66±0.58	8.50 ± 0.43
30 <sup>th</sup>	CB	6.66±0.52	6.66±0.52	7.66±0.52	7.66±0.52	7.16 ± 0.58
	CR	6.66±0.52	6.33±0.52	6.00±0.82	6.33±0.52	6.33 ± 0.27
60 <sup>th</sup>	CB	6.33±0.52	6.00±0.00	6.33±0.52	6.66±0.52	6.33 ± 0.27
	CR	5.33±0.52	5.33±0.52	5.66±0.52	5.00±0.82	5.33 ± 0.27
90 <sup>th</sup>	CB	6.00±0.00	6.00±0.82	5.33±0.52	6.33±0.52	5.91 ± 0.42
	CR	4.66±0.52	5.33±0.52	5.00±0.82	4.66±0.52	4.91±0.32
120 <sup>th</sup>	CB	4.33±0.52	4.66±0.52	4.33±0.52	4.00±0.00	4.33 ± 0.27
	CR	3.66±0.52	4.00±0.82	4.33±0.52	3.66±0.52	3.91 ± 0.32

\*Results are mean of six (n=6) determinations with s.d.

**Table No. 30.** Changes of organoleptic qualities of potassium sorbate treated (T<sub>1</sub>) samples during storage study

Days	Species with treatments	Colour and appearance	Odour	Texture	Fish Flavour Intensity	Overall acceptability
0th	T <sub>1</sub> B	8.66±0.52	8.66±0.52	8.66±0.52	9.00±0.82	8.75 ± 0.17
	T <sub>1</sub> R	8.00±0.00	8.66±0.52	8.33±0.58	8.66±0.50	8.41 ± 0.32
30th	T <sub>1</sub> B	7.66±0.52	8.00±0.82	7.33±0.52	8.66±0.52	7.91± 0.57
	T <sub>1</sub> R	7.33±0.52	8.00±0.00	7.66±0.52	8.66±0.52	7.75 ± 0.57
60th	T <sub>1</sub> B	7.66±0.52	7.00±0.82	7.33±0.52	8.00±0.82	7.5 ± 0.43
	T <sub>1</sub> R	7.00±0.82	7.66±0.52	7.00±0.00	8.00±0.00	7.42 ± 0.47
90th	T <sub>1</sub> B	7.33±0.52	7.33±0.52	7.00±0.82	7.33±0.52	7.25 ± 0.17
	T <sub>1</sub> R	7.00±0.00	7.33±0.52	6.66±0.52	7.33±0.52	7.08 ± 0.32
120th	T <sub>1</sub> B	6.66±0.52	6.66±0.52	5.66±0.52	6.66±0.52	6.42 ± 0.50
	T <sub>1</sub> R	6.33±0.52	6.33±0.52	5.66±0.52	6.33±0.52	6.16 ± 0.79

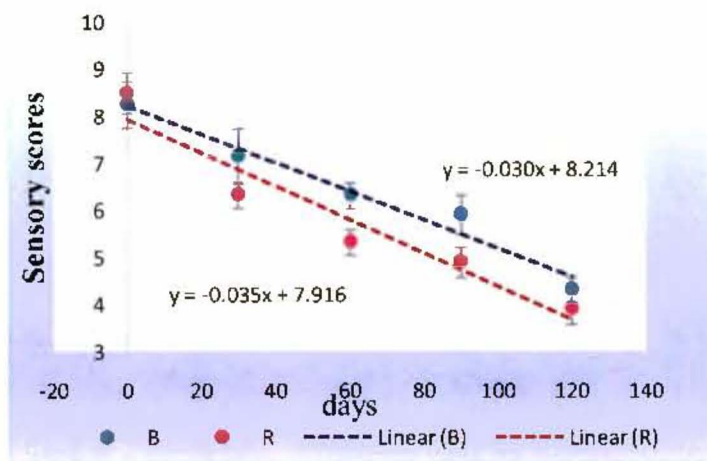
\*Results are mean of six (n=6) determinations with s.d.

**Table No. 31.** Changes of organoleptic qualities of sodium benzoate treated (T<sub>2</sub>) samples during storage study

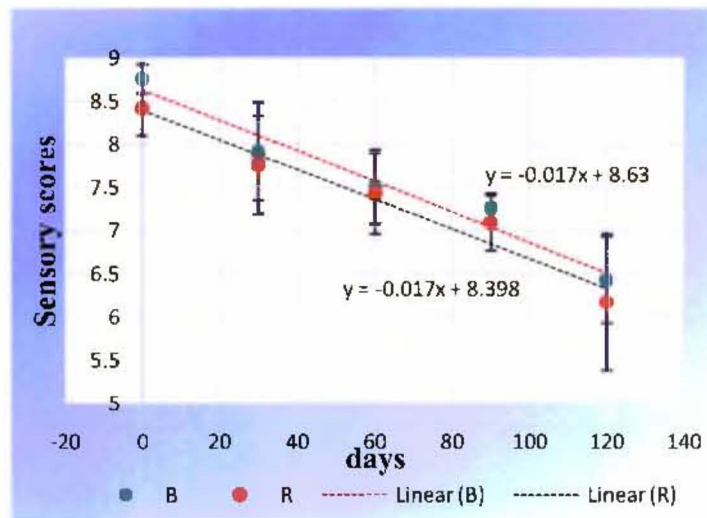
Days	Species with treatments	Colour and appearance	Odour	Texture	Fish Flavour Intensity	Overall acceptability
0th	T <sub>2</sub> B	8.66±0.52	8.33±0.52	9.00±0.82	8.66±0.52	8.87 ± 0.27
	T <sub>2</sub> R	9.00±0.00	8.66±0.52	9.00±0.00	8.33±0.52	8.75 ± 0.32
30th	T <sub>2</sub> B	7.66±0.52	8.00±0.82	8.00±0.82	8.00±0.00	7.91 ± 0.17
	T <sub>2</sub> R	7.33±0.52	7.66±0.52	8.33±0.52	8.33±0.52	7.91 ± 0.74
60th	T <sub>2</sub> B	7.00±0.82	7.66±0.52	7.33±0.52	7.66±0.52	7.14 ± 0.32
	T <sub>2</sub> R	7.33±0.52	7.33±0.52	7.66±0.52	7.33±0.52	7.41 ± 0.17
90th	T <sub>2</sub> B	7.33±0.52	6.66±0.52	6.66±0.52	7.33±0.52	7.00 ± 0.38
	T <sub>2</sub> R	7.33±0.52	6.66±0.52	7.33±0.52	7.00±0.00	7.08 ± 0.32
120th	T <sub>2</sub> B	6.00±0.00	6.00±0.82	6.00±0.82	6.33±0.56	6.08 ± 0.17
	T <sub>2</sub> R	6.66±0.56	5.66±0.52	5.33±0.56	7.00±0.82	6.17 ± 0.79

\*Results are mean of six (n=6) determinations with s.d.

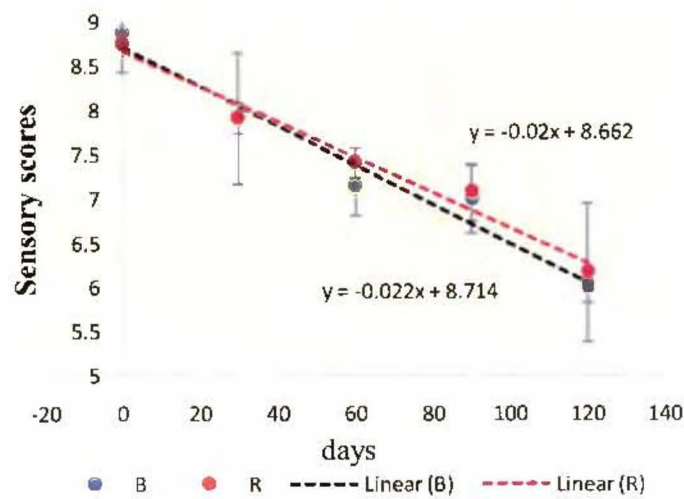
**Fig No. 26.** Changes in overall acceptability of dry fishes of C sample



**Fig No. 27.** Changes in overall acceptability of dry fishes of T<sub>1</sub> sample



**Fig No. 28.** Changes in overall acceptability of dry fishes of T<sub>2</sub> sample



**Plate No. 21. Estimation of Crude fat**



**Plate No. 22. Estimation of TBA by using spectrophotometer**



**Plate No. 23 & 24. Fungal colonies after 5 days incubation period**





*Chapter - 5*

**DISCUSSION**

The khoti is a community-based organisation that during the full fishing season manages and governs the fishing activity, settles disputes and tries to take care of the needs and grievances of its members – the fishers and fish workers. The khoti, in West Bengal is the fishing camp that comes up at the landing centre and manages it for the duration of the full fishing season – roughly from July to February every year. The present study was conducted considering the importance of the organisation through which the main fishing activity on the coast takes place.

The information pertaining to this industry is rather not available or scanty and therefore it was felt necessary to make a thorough study so as to derive a definite conclusion and suggest measures for further development.

### 5.1. Available fishes in different khotis:

The most important fishes available in the study area include *Harpodon nehereus*), Ribbon fish (*Lepturacanthus savala*), *Setipinna phasa* along with other varieties of fishes and average size of the fishes are given in Table. 4. However, the size of other fishes available was not measured because of small size.

#### 5.2.1. Process of dry fish production: (Junput Khoti)

The fishes generally landed in this khoti are Bombay duck (*Harpodon nehereus*), Ribbon fish (*Lepturacanthus savala*), *Setipinna phasa*, *Nandus nandus*, croakers and other small crabs and prawns. Most of the fishers have their own boat or crafts (mostly motorised) and nets (Behundi jaal) (Table. 5). The fishing starts in early hours of the day and return to the landing centre by evening and there are some fishermen who go for fishing in the evening and return by early morning depending on the tide (High and low tide). The average size of fishes harvested is given in Table 4. But some of the people involved in drying activity, bring fishes from Digha-mohona or Shankarpur fish landing centre. The fishes harvested in these landing centres, are relatively large in size because the fishing involves large trawlers using trawl nets. A few women of this khoti have developed a Women's co-operative named Sebika Matsya Samabai Samity under Fisheries Department, Govt. of West Bengal who dry the fishes using solar drier without any treatment.

Generally Bombay duck and ribbon fishes are dried by hanging from bamboo pole or structure, whereas *Setipinna phasa*, croakers (*Johnius* spp.), small prawns and crabs are dried on mats or raised wooden platform. In Junput khoti, dip treatment in 1000 lt of water with 3lt of chemical and 50 kg of salt for 1 ton of fish is done before

drying. The dip treatment of fishes has certain advantages like: a) it will extract the intercellular water from the fish body helping to reduce the period of drying, b) the dry fishes will be free from any fungal attack, fly or beetle infestation, c) the appearance and the flavour of these dry fishes give the consumer better acceptability. The buyers either buy the dried fishes from the khoti directly or the fishes are sold to Balighai dry fish market near Egra, Purba Medinipore.

### 5.2.2. Process of dry fish production: (Jalda Khoti):

This khoti is different from Junput khoti as there is no dip treatment given to the fishes prior to drying. They simply dry the fishes after harvesting either by spreading over the mat or by hanging them in open sun. The fishes generally dried in this khoti are Bombay duck (*Harpodon nehereus*), Ribbon fish (*Lepturacanthus savala*), *Setipinna phasa*, *Chanda nama* and many other small crabs and prawns (Table 5). Fishers have their own boat or crafts (mainly motorised) and nets (Behundi jaal) used for fishing.

The buyers either buy the dry fish directly on contact basis or are sold to Balighai dry fish market near Egra, Purba medinipore. The season of drying are generally continued from October to mid-March. The women of these khotis are involved in other activities other than drying like making prawn pickles, fish silages, and sausages etc. which helps to earn extra revenue during off season.

### 5.3. Proximate composition and Quality parameters of dry fishes of the study area:

#### 5.3.1. Proximate Composition:

##### 5.3.1.1. Protein content:

The mean protein value of dry Bombay duck and Ribbon fish at Jalda khoti was  $58.39\% \pm 1.08$  and  $61.19\% \pm 0.41$  respectively (Table. 6) (Fig.2). Similarly, the value for Junput Khoti was  $57.08\% \pm 1.72$  and  $61.67\% \pm 1.39$  in the same order (Table. 9) (Fig.5). Rahman *et al.*, (1982) observed 55.75% to 64.49% protein level in dried marine fishes. Siddique and Majumder (2012) found that the mean percentage of protein of dried Bombay duck and Ribbon fish varies in the range of 52.44% to 71.32%. The results of this study are in concurrence with the earlier findings.

### 5.3.1.2. Lipid content:

The observation revealed that mean fat content of dry Bombay duck and Ribbon fish at Jalda khoti was  $18.22\% \pm 1.14$  and  $15.37\% \pm 0.86$  respectively (Table. 6) (Fig.2). Similarly, mean fat content at Junput khoti was  $16.15\% \pm 1.22$  and  $12.97\% \pm 0.39$  for Bombay duck and Ribbon fish (Table. 9) (Fig.5). Rubbi *et al.*, (1987) reported that the lipid content ranges from 0.45 to 15.51% in dried marine fishes. Lovern (1950) reported that the variation in lipid content could be influenced by variation among species, diet, temperature, salinity, selective mobilization and distribution.

### 5.3.1.3. Moisture content:

The percentage of moisture found in dry Bombay duck and Ribbon fishes at Jalda khoti was  $7.24\% \pm 0.87$  and  $8.08\% \pm 1.17$  (Table. 6) (Fig.2) and the values for Junput khoti was  $11.81\% \pm 0.55$  and  $17.41\% \pm 1.39$  (Table. 9) (Fig.5) respectively. Bhuiyan (1992) observed 6.9% to 14.2 % moisture in dry Bombay duck and Sin croakers. Pravakar and Mansur (2013) reported that moisture content of dried Ribbon fishes was  $23.94 \pm 0.2\%$  (where highest and lowest value varies from 23.73% to 24.21%) whereas the moisture content of dried Bombay duck was  $27.19 \pm 0.27\%$ . The present findings with regard to moisture content are in concurrence with the results of the above author. The initial moisture content is the most important factor affecting the ultimate quality of dried fish. Relekar *et al.*, (2014) reviewed that the initial moisture content of dried ribbon fish procured from market was 30.6 % which was significantly higher than ribbon fish dried in solar tent drier (17.85%). Moisture content of the final product should be reduced to less than 15-16% where most of the microbiological and enzymatic activities are slowed down (Pravakar *et al.*, 2013). The moisture content of two species are found relatively high because of its absorption after drying during storage in gunny bags in wet atmosphere. MPEDA has specified 35% as the maximum moisture level for dried ribbon fish (MPEDA, 2003). The moisture content of dry fish collected from Junput and Jalda khoti are well within the specified limit of moisture.

### 5.3.1.4. Ash content:

In the present study, the ash level of analysed dry Bombay duck and Ribbon fish of Jalda khoti was  $10.78\% \pm 0.23$  and  $14.69\% \pm 0.4$  (Table. 6) (Fig.2) respectively,

whereas the mean value for Junput khoti were  $11.29 \pm 0.19$  and  $13.06 \pm 0.72$  (Table No. 9) (Fig.5). Gheyasuddin *et al.*, (1980) found (4.56-9.98) % ash content in some marine dried fish samples which fairly match with the present investigations. According to Relekar *et al.*, (2014), the initial ash content of the dried ribbon fish procured from the market was lower (15.35%) which is in concurrence with the findings of the present experiments.

### 5.3.2. Biochemical Parameters:

#### 5.3.2.1. Total Volatile Base Nitrogen (TVBN):

TVBN basically comprise of low molecular weight volatile metabolic products generated especially during post mortem bacterial action and one of the indices of post mortem spoilage. A lower TVBN value indicates good quality of the products and a high TVBN value correlate with the high bacterial activity and high rate of spoilage, which results in unacceptability of the products for human consumption (Joseph *et al.*, 1983).

The results of the experiment shows the mean values for dry Bombay duck and ribbon fish were  $(40.59 \pm 3.01)$  mg % and  $(80.27 \pm 3.01)$  mg% (Table. 7) (Fig.3) at Jalda khoti and for Junput khoti, the values were  $(26.55 \pm 2.35)$  mg% and  $(65.9 \pm 4.69)$  mg% (Table. 10) (Fig.6) respectively. According to Immaculate and Sinduja (2013), the highest TVB-N values were observed in dry fishes of Tuticorin fishing villages during monsoon season which varied from 20.28 to 38.26 mg/100 g. Islam (2001) observed that TVBN content of traditional dried ribbon fish, Bombay duck, big eye tuna, silver Jew fish and Chinese pomfret was in the range of (16.56-44.83) mg %. Recently Relekar and Joshi (2014) have reported that the TVBN value for dried ribbon fish collected from market was initially 70.6 mg%. The findings of the present experiment are in concurrence with the above results. The acceptable level of TVB-N in fishes is 35-40 mg /100 g (Kimura, 1934). Considering the acceptable limit, the dry Bombay duck of Jalda khoti and the ribbon fishes of both the khotis are considered not fit for human consumption. This is mainly because of bacterial infestation during open drying or due to poor handling and storage methods which helps in bacterial proliferation lead to spoilage. Huss (1995) has reported that TVBN is formed by bacterial de-amination of proteins, peptides and amino-acids.

### 5.3.2.2. Tri-methyl amine (TMA):

It is often used as an index to assess the quality of seafood products (Hebard, 1982). Beatty and Gibbons (1936) have reported that the production of TMA-N depends on the bacterial activity. The TMA value of sun dried Bombay duck and ribbon fish of Jalda khoti were  $(27.73 \pm 0.39)$  mg% and  $(40.32 \pm 1.25)$  mg% (Table. 7) (Fig.3), where the values for same species of Junput khoti were  $(15.06 \pm 1.17)$  mg% and  $(38.82 \pm 3.89)$  mg% (Table. 10) (Fig.6) respectively. Immaculate and Sinduja (2013) observed that the TMA-N value of salted and sun dried fishes of Tuticorin fishing villages ranged between 9.8 – 18.8 mg/100 g during monsoon season and during post monsoon season the value ranged from 6.3 – 17.1 mg/100 g. Connell (1980) has recommended the level of 10-15 mg/100 g of TMA-N for human consumption. In the present study, dried fishes collected from the khoti had the TMA-N value above the acceptable limit. This is mainly because of bacterial proliferation after drying due to unhygienic handling and storage. Mohd. Yusuf Ali *et al.*, (2010) opine that the production of TMA-N was dependent on the bacterial activity as well as from endogenous enzyme.

### 5.3.2.3. Peroxide Value (PV):

Lipid per-oxidation is one of the primary mechanisms of quality deterioration in stored foods, especially in muscle tissues (Gheisari, 2011). The change in Peroxide value is the indicator of measurement of primary lipid oxidation. The PV values of dry Bombay duck and ribbon fish of Jalda khoti was found  $(19.1 \pm 4.94)$  mili-equi O<sub>2</sub>/ kg fat and  $(11.82 \pm 6.42)$  mili-equi O<sub>2</sub>/ kg fat (Table. 7) (Fig.3) but for Junput khoti, the values were  $(5.24 \pm 0.21)$  and  $(34.72 \pm 2.99)$  mili-equi O<sub>2</sub>/ kg fat % (Table 10) (Fig.6) respectively. Debnath and Majumder, (2013) reported that PV values of market samples of *P. sophore* and *M. gulio* was 26.33 and 31.45 respectively. Connell (1976) has suggested that if PV is above 20 milli moles of oxygen per kg of fat, then the fish may show off odour and taste rancid. The results of the present experiment revealed that the PV value of dry ribbon fishes of Junput khoti shows values beyond acceptable limit. The PV values of ribbon fishes of Jalda khoti show lower value than that of Junput khoti. This may be because of more lipid oxidation in Ribbon fishes of Jalda khoti that reduces the peroxide value and leads to the production of carbonyls and other secondary oxidation products which impart the characteristic rancid off flavour

to the product. Similarity was found in case of dry Bombay duck at Junput khoti shows lower PV value with higher TBA value (Table No.10) than that of Jalda khoti. The oxidation of fat during drying may lead to rancid flavour (Tsuchiya, 1961).

#### 5.3.2.4. Thio-barbituric Acid (TBA):

TBA is widely used as indicators for the assessment of degree of secondary lipid oxidation. This TBA factor is responsible for rancid flavour, off odours, colour as well as texture deterioration and nutritional value (Olafsdottir *et al.*, 1997). The TBA value obtained for dry Bombay duck and ribbon fishes of Jalda Khoti were 3.61 mg MDA/kg fat and 10.08 mg MDA/kg (Table. 7) (Fig.3) fat respectively. The values were different in case of Junput khoti, i.e. 4.52 mg MDA/kg fat and 8.39 mg MDA/kg fat (Table. 10) (Fig.6) for dry Bombay duck and ribbon fishes respectively. It has been suggested that a maximum TBA value, indicating good quality of fish is 5 mg malonaldehyde/kg while fish may be consumed up to a TBA value of 8 mg malonaldehyde (MDA)/kg (Schormuller, 1969). The results of the present experiment show that the values of TBA was beyond acceptable limit for ribbon fish of the both the khotis (Table No. 7 and 10). The fat content in Ribbon fish is relatively more in comparison to Bombay duck and in this case, fraction of lipid oxidation was more due to traditional drying practices.

#### 5.3.3. Microbiological characteristics:

##### 5.3.3.1. Total Plate Count:

Microbiological analysis also showed variations among the samples. The total plate count expressed as colony forming unit in one gram of sample (CFU/g) of the representative samples of dry Bombay duck and ribbon fish of different khotis. The bacterial load of Bombay duck and ribbon fishes of Jalda khoti was found  $1.17 \times 10^7$  CFU/g (log value 7.067) and  $2.79 \times 10^7$  CFU/g (log value 7.445) (Table.8) (Fig.4). For similar species in Junput khoti, the value were  $1.008 \times 10^6$  CFU/g (log value 6.003) and  $1.55 \times 10^7$  CFU/g (log value 7.165) respectively (Table. 11) (Fig.7). Pravakar (2013) has reported that bacterial load of dried Chinese Pomfret, Bombay duck and Ribbon fish were  $3.8 \times 10^5$ ,  $3 \times 10^4$  and  $2.1 \times 10^5$  CFU/g respectively with lowest value in Bombay duck and highest value in Chinese Pomfret. Basu *et al.*, (1989) have reported that TPC value of dried ribbon fish collected from market of Andhra Pradesh was higher ( $4.2 \times 10^4$  cfu/g) than laboratory dried and tunnel dried ribbon fish. In fresh

fish, the acceptable limit is  $5 \times 10^5$ /g at  $37^\circ\text{C}$  but for cooked or dried fish, the permissible limit is  $1 \times 10^5$ /g at  $37^\circ\text{C}$  (Surendran, 2006). The values in the present experiment are quiet higher than the acceptable limit which could be due to unhygienic drying and storage practices leading to post process bacterial contamination and spoilage.

### 5.3.3.2. Total Fungal Count:

The quality of salted and sun dried fishes is adversely affected by the presence of different types of fungi in fish and fishery products (FDA, 1982). The results of the fungal counts in sun dried fishes (Bombay duck and ribbon fishes) of Jalda khoti was  $1.08 \times 10^3$  CFU/gm (log value 3.027) and  $9 \times 10^2$  CFU/gm (log value 2.954) respectively (Table.8) (Fig.4). Similarly the value for Junput Khoti was  $9.9 \times 10^4$  CFU/gm (log value 4.994) and  $6 \times 10^4$  CFU/g (log value 4.772) for the same order (Table. 11) (Fig.7). Prakash and Jeyasanta (2011) have reported that fungal counts were high in sun dried sea foods during monsoon season. *S. fimbriata* had the highest fungal count ( $8 \times 10^3$  cfu/g) followed by *S. commerson* ( $5 \times 10^3$  cfu/g), *S. lysan* and *K. Pelamis* ( $4 \times 10^3$  cfu/g), *S. commersonii* ( $3 \times 10^3$  cfu/g) and *A. indicus* ( $2 \times 10^3$  cfu/g). The results of the present experiment corroborate with the findings of Prakash and Jeyasanta (2011). As observed, the fungal count is on higher sides in both the species at Junput khoti as compared to Jalda. The higher fungal count in case of Junput khoti could be because of the higher moisture content in the species and probably because of humid condition existing at Junput compared to Jalda khoti. Patterson and Ranjitha (2009) reported that the total fungal count was high in commercially sun dried fish sample due to high moisture content and humidity. The cured fish in hot humid tropical climates are liable to deteriorate during storage due to mould growth.

### 5.4. Comparative study in quality of dry fish (Bombay duck and Ribbon fish) collected from two khotis:

The results of the experiment revealed that the mean values of quality parameters such as TVBN and TMA of dry Bombay duck and ribbon fish at Jalda khoti (Table.7) (Fig.3) shows higher value compared with the same species (i.e. Bombay duck and ribbon fish) of Junput khoti (Table. 10) (Fig. 6). This is because the production of TMA and TVBN are dependent on the bacterial activity (Mohd. Yusuf and Sharif, 2010) and the treatments (i.e. dip treatment in salt and chemical solution)

followed prior to drying at Junput khoti, reduces the chances of microbial spoilage and extends the shelf life of the dried products. In case of Jalda khoti, there is no such treatment given which results in loss of quality during traditional sun drying process. The PV value of both Bombay duck and ribbon fish (Table. 7) in Jalda khoti shows less value than Junput khoti (Table. 10) because of more lipid oxidation and rancidity resulting in higher TBA value.

The preservative action of salt and chemical solution used prior to drying of fishes in Junput khoti, had resulted low bacterial proliferation in dried fishes, whereas, in Jalda khoti, the fishes are dried openly without any pre-treatment which adversely affects the microbial quality of the dry fishes. (Table.8). Ismail and Wootton (1982) have opined that salt has bacteriostatic and bactericidal effect and it also exerts strong antimicrobial effect during post process storage.

Total fungal count is directly co-related with moisture content of the product (Rao, 1962). The TFC value of Junput dry fishes had quiet higher range (Table. 11) (Fig.7) than that of Jalda khoti (Table No.8) (Fig. 4). This may due to higher moisture content of the dry fishes of Junput khoti. There is a tendency in our country that fish processors sometimes allow more moisture in dried fish products to gain weight for economic benefit. In a tropical country like India, where relative humidity is always high and there is a chance of uptake moisture from the environment. Excessive moisture uptake increases the water activity which facilitates the growth of micro-organisms and reduces the loss of nutrient and shelf life of dried products.

## 5.5. Studies on the quality of raw material:

### 5.5.1. Physical characteristics of raw material:

The total length and weight of fresh Bombay duck was found in a range between (20-27) cm and (33-107) grams from Kakdwip market which corroborates with the findings of Kumar and Reddy (2014). Similarly, the length of ribbon fishes taken for experiment were in the range of (46-60) cm and weight varied from (70-101) grams. James *et al.*, (1967) have reported that in commercial fishery, specimens of *Lepturacanthus savala* were recorded in the range of 200-700 mm length. Length of the specimens of *Lepturacanthus savala* collected from Ratnagiri coast ranged between 100-700 mm (Pallavi *et al.*, 2013). The experimental results of Kudale and Rathode, (2014) pointed out that the average weight of *Lepturacanthus savala* of both male and female varied from 58.2 g to 335.6 g at Karnataka state.

### 5.5.2. Biochemical characteristics of raw material:

The proximate composition of fresh Bombay duck (*Harpodon nehereus*) and Ribbon fish (*Lepturacanthus savala*) are given in (Table. 12) which corroborates with the findings of Kumar *et al.*, (2012).

Sernapesca (2001) had reported that the maximum permitted level of volatile amines are 15 mg TMA/ 100g fish and 30 mg TVBN/ 100g fish for raw fish. In the present experiment, the values of TMA and TVBN of both the species (Table.13) were within this range. According to Beatty and Gibbons (1936), TMA-N and TVB-N values indicate freshness of the fish. So the results obtained in present study indicate that the raw fishes used were of good quality.

Peroxide value (PV) is regarded as a reliable method to estimate the extent of auto oxidation in the early stage (Olley and Lovern, 1960). The results of the present experiment shows that the PV value of fresh Bombay duck and ribbon fish were  $1.69 \pm 2.39$  mili-equi O<sub>2</sub>/kg fat and  $10.51 \pm 0.64$  mili-equi O<sub>2</sub>/kg fat (Table.13) respectively. Connell (1976) has suggested that if PV is above 20 milli -moles of oxygen per kg of fat, then the fish may show off odor and taste rancid. Therefore it may be concluded that the selected fishes were of good quality.

Surendran *et al.*, (2006) mentioned that the acceptable limit of TPC for microorganisms per gram weight fresh fish is  $5 \times 10^5$ /g at 37°C. In our present study, the TPC values of fresh Bombay duck and Ribbon fish were found  $2.18 \times 10^4$  CFU/g and  $2.485 \times 10^4$  CFU/g (Table. 14) respectively indicating that the samples collected for the experiment were of good quality. The present study shows no fungal growth on both the fishes.

### 5.6. Curing of fish before drying:

The fishes were subjected to two different types of curing treatments prior to drying in open sun. One lot was treated with dry salt and 0.25 potassium sorbate (T<sub>1</sub>) and another batch was treated with saturated brine and 0.3% sodium benzoate (T<sub>2</sub>) for over-night (12 hrs). The control sample (C) was treated with saturated brine. Safos and Busta (1993) have reported that effective microbial concentration of sorbic acid in most foods is in the range of 0.02-0.30%. To be effective against mould growth, potassium sorbate should be incorporated at a level of 2 % on the weight of common

salt (when salt to dressed weight of fish is 1:8, i.e. for light salting) and at 0.5% for 1:4 (heavy salting) (Sen and Rangaswami, 1966). Corresponding figures for sodium benzoate to be effective against red halophiles is 0.5% and 0.25%. Chakrabarti, (2009) reported that the sorbate level in fish muscle decreased on storage. Thus, a dip in 0.2 % potassium sorbate solution for 2 h could be recommended to maintain residual sorbate level below the accepted permissible limit of 1000 ppm for all five species to control fungi during storage even upto 4 - 6 months at ambient temperature. Common salt (both in the form of dry salt and brine solution) is used as curing agent where potassium sorbate and sodium benzoate has been used to control the microbial growth of the samples which in turn will increase the shelf life of the product. Uniformity was maintained with regard to period of treatment as it is being practiced in the field (Study area).

#### 5.7. Drying of fish:

During the drying process, a significant reduction ( $p < 0.05$ ) in moisture content was observed in all the treatments including control over a period of 10 days (Table 15). The minimum moisture content (6.9 %) was observed in T<sub>2</sub> sample of Bombay duck showing a reduction of almost 80 % moisture. However in case of ribbon fish the controlled sample (C) recorded the least figure (8.11 %) for moisture content exhibiting a reduction by almost 56 %. Frazier and Westhoff (1978) have reported that no microbe (yeast, mould and bacteria) can grow in a product with moisture content below 15%. Analysis of variance among the different treatments reveals no significant ( $p > 0.05$ ) influence of potassium sorbate and sodium benzoate on drying process in relation to moisture content.

#### 5.8. Proximate Composition of the fishes after drying:

##### 5.8.1. Protein content:

The experimental results show no such difference on the proximate composition of Bombay duck and Ribbon fishes between two different treated samples (T<sub>1</sub> and T<sub>2</sub>) and the control sample (Table. 16) (Fig.12 and 13). The values of crude protein for dry Bombay duck ranges from 53.91 to 56.33 % and in case of ribbon fish, the value ranges from 52.33 to 53.76 %. Siddique and Aktar (2011) have reported similar trend giving value for crude protein 58.33 % $\pm$ 0.4 for freshly dried *Harpodon nehereus*. Relekar *et al.*, (2014) found that the ribbon fish dried in solar

tunnel drier recorded relatively higher protein content (44.13%) right from the beginning of storage, followed by fish dried on raised bamboo platform (42.22%) and on black polythene sheet (41.35%) whereas Siddique and Aktar (2011) have reported that the mean protein percentage of freshly dried ribbon fish was  $(71.90 \pm 0.25)$  %. The result of this experiment is in concurrence with the findings of the earlier work.

In case of Bombay duck, the sodium benzoate treated samples had higher protein (56.33 %) than the controlled one (53.97%) (Table 16) (Fig.12). Arekemase *et al.*, 2012 have reported that tilapia treated with such preservatives had higher protein content as against the control sample.

#### 5.8.2. Fat content:

The experimental result shows that the fat content of freshly dried Bombay duck ranges from 5.94 to 11.77% and for ribbon fish 11.53 to 13.79 % (Table. 16) (Fig. 12 and 13). The values of this experiment are quite low. Siddique and Aktar (2011) have observed that the mean values of freshly dried Bombay duck and ribbon fish were  $(7.78 \pm 0.50)$  % and  $(7.79 \pm 0.11)$  % respectively and the values on higher side could be because of more lipid oxidation resulting in increased PV and TBA values.

#### 5.8.3. Ash content:

The mean values of ash content for dry Bombay duck and Ribbon fish of different treatments were observed 14.78 to 21.96 % and 17.86 to 23.00 % (Table. 16) (Fig. 12 and 13) respectively. Haque and Kamruzzaman (2013) have reported that the mean ash percentage of traditional sun dried Bombay duck was 16.95% to 21.41 %. Relekar *et al.*, (2014) have reported that the initial ash content of the dried ribbon fish procured from the market was lower (15.35%) as compared to the fishes dried in solar tunnel drier (16.67%), on raised bamboo platform (16.73%) and on black polythene sheet (16.70%). The present experiment shows little higher value which could be due to salt content, indicating that the higher proportion of ash is mostly due to salt treatment during processing of ribbon fish (Khuntia, 1990). The result of Khuntia *et al.*, (1990) revealed a high degree of positive correlation ( $r = 0.9989$ ) between ash and salt content.

#### 5.8.4. Moisture content:

The percentage of moisture found in dry Bombay duck and Ribbon fishes of different treatments varied from 6.9 % to 7.42 % and 8.11 % to 17.59 % respectively (Table. 16) (Fig. 12 and 13). Clucas (1982) reported that the dried fish with 25% or more moisture is not sufficient to inhibit microbial growth whereas dried fish with 15% or less moisture is good enough to inhibit microbial growth. Similar cut-off point was also reported by Fraizer (1978). Moisture content in terms of water activity influences the microbial growth and hence, affects the stability of food during storage (Arekemase, 2012). So, in the present experiment, the drying had been continued until the moisture content was reduced to a certain limit, which may increase during subsequent storage but will not increase to the level to influence the microbial proliferation.

#### 5.9. Changes in Biochemical and Microbiological Parameters during storage:

Storage study was done of both samples (*Harpodon nehereus* and *Lepturacanthus savala*) treated with different preservatives to assess the changes in quality of these products during its storage period.

##### 5.9.1. Total Volatile base Nitrogen (TVBN):

Total volatile base nitrogen contains total amount of volatile nitrogen bases (Trimethyl-amine) together with nitrogen which is synthesized from protein and mainly as a result of microbial activity (Connell, 1975). The TVBN values of T<sub>1</sub>, C, and T<sub>2</sub> samples were increased during storage period which is in concurrence with the result of Chakrobari (2009) and Relakar (2014). For T<sub>1</sub>, C and T<sub>2</sub> treatments, the value for Bombay duck increased significantly ( $p < 0.05$ ) from 26.09 mg % to 48.87 mg % and 24.79 mg % to 41.18 mg% and from 19.67 mg% to 29.94 mg% (Table. 17) (Fig.14) respectively. For ribbon fish, same significant ( $p < 0.05$ ) increasing trend was observed in both the treatments and control sample (i.e. T<sub>1</sub>: 13.25 mg % to 42.52 mg %, C: 12.28 mg % to 49.53 mg% and T<sub>2</sub>: 12.84 mg% to 36.49 mg %) (Table. 18) (Fig.20) respectively. Adebona (1978) has reported that TVBN produced by microbial degradation of nitrogen tissue components, was found to increase steadily in all the products. The initial TVBN values of all three samples (i.e. T<sub>1</sub>, C and T<sub>2</sub>) were less than the values found by Pravakar (2013) for the same species. However, the results

show significantly lower values ( $p < 0.05$ ) in  $T_1$  and  $T_2$  samples with preservatives as compared to control. This may be due to antimicrobial activity of these preservatives retarding microbial activity. The acceptable level of TVB-N in fishes is 35-40 mg/100 g (Kimura, 1934). The experimental result also highlights that the dry Bombay duck of  $T_1$  and C samples reached beyond acceptable limit after a period of 90 days (Table. 17) (Fig.14) but the  $T_2$  samples remained in good condition throughout the storage period. Similarly, for dry ribbon fish of  $T_1$  and  $T_2$  samples were found unacceptable after a period of 90 days but sample C became unfit after 60 days of storage (Table. 18) (Fig.20).

### 5.9.2. Tri -methyl Amine (TMA):

The TMA value increased in all the samples (Table. 19 and 20) (Fig.15 and 21) and such similar observation was also reported by Khuntia (1990). During the storage period, the value for dry Bombay duck in  $T_1$ , C and  $T_2$  samples increased significantly ( $p < 0.05$ ) upto 24.82 mg%, 19.53 mg% and 15.24 mg% respectively. In case of Bombay duck, the use of preservatives do not seem to have any significant influence ( $p > 0.05$ ) in controlling microbial activity resulting in similar increase in TMA values within the treatments. It may also be because of variation in TMAO content among the species concerned. In case of ribbon fish, values increased significantly ( $p < 0.05$ ) upto 23.11 mg%, 23.11 mg%, 16.59 mg% for  $T_1$ , C and  $T_2$  samples respectively at the end of the storage period. Significant ( $p < 0.05$ ) lower values of TMAN in products with preservatives could be because of the preservative effects of the additives in retarding microbial activity. However, the values of TMA of control samples strongly correlate (BD:  $r = 0.967$ ; RF:  $r = 0.998$ ) with the values of total bacterial counts in case of both the species during storage. But the degree of correlation was lower in case of preservative treated  $T_1$  and  $T_2$  samples. Preservatives used in salted fish retard microbial growth and activity by inhibiting various enzymes in microbial cells, especially those in the citric acid cycle (Sen and Lahiry, 1962; Lueck, 1980). Higher values observed in wet salted products may be attributed due to increased microbial load. Mohd. Y. Ali *et al.*, (2010) opined that the production of TMA-N was dependent on the bacterial activity as well as from endogenous enzyme. The recommended level of the TMA-N value for human consumption is 10-15 mg/100g (Connell, 1980). The experiment concludes that the TMA values for dry Bombay duck of  $T_1$  and C sample reached to threshold limit after a period of 60 days

(Table. 19) (Fig.15) where the T<sub>2</sub> sample continued to be in good condition at the end of 90 days storage. Similar trend was also found in case of ribbon fish also (Table. 20) (Fig. 21).

### 5.9.3. Peroxide Value:

PV recorded an increase in all the dry samples, which is supported by the findings of Kumar (1997). The values for both dry Bombay duck and ribbon fish for T<sub>1</sub>, C and T<sub>2</sub> samples increased significantly ( $p < 0.05$ ) from 6.88 to 35.32 and 8.46 to 51.22 mili-equi O<sub>2</sub>/ kg fat respectively (Table. 21 and 22) (Fig.16 and 22). However, use of preservatives do not seem to have any significant influence ( $p > 0.05$ ) in controlling lipid oxidation in medium fatty fish like ribbon fish resulting in similar increase in peroxide values within the treatments and the control sample. Increase of peroxide value may be attributed to the oxidation of highly unsaturated fatty acids in fish lipids by the catalytic activity of common salt (Amano, 1962), pro-oxidant action of moisture and also auto oxidation by atmospheric oxygen (Wheaton and Lawson, 1985). The result indicates that the salting and drying conditions accelerate lipid oxidation and this is in agreement with the results of previous workers (Waterman, 1976; Smith, 1988; Smith *et al.*, 1988). This may be the main reason for all the samples of three different treatments showing increasing trend of PV with respect to time. It has been observed that, in case of T<sub>2</sub> sample, the values of PV in ribbon fishes (Table. 22) (Fig.22) increased initially but decreased sharply at later stage. Connell (1976) has suggested that if PV is above 20 mili-moles of oxygen per kg of fat, then the fish may show off odour and taste rancid. The PV of dry Bombay duck and ribbon fish of C sample were considered acceptable upto 90 days and 30 days of storage period respectively (Table. 21 and 22), whereas the Bombay duck of T<sub>2</sub> sample was acceptable throughout the storage period but the ribbon fishes became unacceptable only after 30 days. The PV of both the dry Bombay duck and ribbon fishes of T<sub>1</sub> samples were acceptable upto 30 days. This increasing trend of PV in case of T<sub>1</sub> sample may be due to of dry salting. It may possible that the iron impurities present in crude salt may be helping to accelerate the rate of lipid oxidation. However, the rate of fat deterioration was very gradual. Fish oil has been found to be more liable to spoilage than other oils due to their greater number of unsaturated fatty acids (Eyo, 1993).

#### 5.9.4. Thio-birbituric acid (TBA):

The TBA value of all the samples were increased significantly ( $p < 0.05$ ) during storage period. At the end of storage period, the TBA values for dry Bombay duck of all treatments ( $T_1$ , C and  $T_2$ ) were increased upto 7.36mg MDA/kg, 3.79 mg MDA/kg and 6.27 mg MDA/kg respectively (Table. 23) (Fig.17). Similarly, the values for ribbon fishes were increased upto 7.30 mg MDA/kg, 7.33mg MDA/kg and 8.22 mg MDA/kg (Table. 24) (Fig.23) for  $T_1$ , C and  $T_2$  samples. Analysis of variance among the different treatments reveals no significant ( $p > 0.05$ ) influence of potassium sorbate and sodium benzoate on secondary lipid oxidation and production of TBA values in ribbon fish. However, in case of Bombay duck, the result shows significantly higher value ( $p < 0.05$ ) in  $T_1$  and  $T_2$  samples than the control one. The level of increase was more in case of ribbon fish during storage period. (Table. 24) (Fig.23). TBA is widely used as an indicator for the assessment of degree of secondary lipid oxidation. It has been suggested that a maximum TBA value indicating acceptable quality of the fish is 5 mg malonaldehyde/kg while fish may be consumed even up to 8 mg malonaldehyde (MA)/kg (Schormuller, 1969). Several other authors (Karacum and Boran, 1996) have reported that TBA value above 3-4 malanoaldehyde/ kg indicates quality loss in the product. Bernardez (2005) stated that double bonds of unsaturated fatty acids are highly susceptible to oxidation and this leads to the production of carbonyls and other secondary oxidation products which impart the characteristic rancid off flavour to the product.

#### 5.9.5. Total Plate Count (TPC):

Throughout the period of storage, TPC shows significant ( $p < 0.05$ ) increasing trend in all the treatments for both Bombay duck and ribbon fish (Table. 25 and 26) (Fig. 18 and 24) which is well corroborated with the findings of Oksüztepe (2010). During storage, TPC values of dry Bombay duck of  $T_1$ , C and  $T_2$  samples were increased upto  $2.52 \times 10^7$  CFU/gm (log value 7.27),  $4.365 \times 10^8$  CFU/gm (log value 8.63) and  $4.05 \times 10^7$  CFU/gm (log value 7.55) after 120 days storage respectively (Table. 25) (Fig.18). The values for ribbon fishes were higher as compared to Bombay duck (i.e.  $1.98 \times 10^7$  CFU/gm (log value 7.24),  $1.034 \times 10^9$  CFU/gm (log value 8.86) and  $3.24 \times 10^7$  CFU/gm (log value 7.44) respectively) (Table. 26) (Fig.24) for  $T_1$ , C and  $T_2$  samples at the end of the storage period. However the results shows significantly lower value ( $p < 0.05$ ) in  $T_1$  and  $T_2$  samples with preservatives as compared to control

due to the bactericidal effect of preservatives on the microbes. Sodium Benzoate and potassium sorbate have been reported to retard microbial activity by inhibiting various enzymes of the microbial cell, specifically those in the citric acid cycle (Lueck, 1980). Similar observation was made by Oksuztepe (2007) in case of potassium sorbate treated groups containing lower microbial load compared to control group and preservation was related to the antimicrobial effect of potassium sorbate (Safos and Busta, 1981; Yetim, 1996; Patir, 2001). The ICMSF (1986) has considered an aerobic plate count limit of  $10^5$ - $10^7$  (5-7 log cfu/g) for fish that is acceptable for human consumption. The results revealed that, the values of dry Bombay duck and ribbon fish of T<sub>1</sub> and T<sub>2</sub> samples reached to unacceptable limit after a period of 90 days (Table. 25 and 26) during storage period and the total microbial counts for both (T<sub>1</sub> and T<sub>2</sub>) samples were always less than the control one. Investigation by Safos and Busta, (1985, 1993) has revealed that sorbates can inhibit gram-positive and gram – negative, catalase- positive and catalase-negative, aerobic and anaerobic, and mesophilic and psychophilic microorganisms, as well as spoilage and pathogenic bacteria. The values of TPC of dry Bombay duck in C sample is strongly co-related ( $r = 0.975$ ) with the values of TVBN followed by T<sub>1</sub> ( $r = 0.928$ ) and T<sub>2</sub> ( $r = 0.825$ ) samples during the storage period. However, in case of ribbon fishes, the bacterial counts of T<sub>1</sub> sample shows stronger co-relation ( $r = 0.928$ ) with the respective TVBN values than control ( $r = 0.857$ ) and T<sub>2</sub> ( $r = 0.849$ ) samples.

#### 5.9.6. Total Fungal Counts:

Fungal counts were significantly ( $p < 0.05$ ) increased within a period of 120 days storage study among all the treatments (Table. 27 and 28) (Fig.19 and 25). For dry Bombay duck, the values were increased upto  $5.4 \times 10^6$  CFU/gm (log value 6.64),  $3.69 \times 10^6$  CFU/gm (log value 6.56) and  $2.7 \times 10^6$  CFU/gm (log value 6.19) for C, T<sub>1</sub> and T<sub>2</sub> samples (Table. 27) whereas for ribbon fish, the values increased upto  $9.6 \times 10^7$  CFU/gm (log value 7.96),  $6.3 \times 10^6$  CFU/gm (log value 6.622) and  $8.1 \times 10^6$  CFU/gm (log value 6.41) (Table. 28) respectively after a period of 120 days storage period. The results shows significantly lower value ( $p < 0.05$ ) in T<sub>1</sub> and T<sub>2</sub> samples with preservatives as compared to control sample which may be due to antifungal effects of the preservatives. Safos, (1989) has reported that sorbic acid inhibits yeast and mould growth. Ogiehor and Ikenebomeh, (2004) have reported that sodium benzoate

exerted high antimicrobial effect on the growth, survival and aflatoxin production ability of *A. niger*, *A. flavus* and *A. fumigatus*. The rapid reduction in the water activity ( $a_w < 0.75$ ) is the most important factor in controlling fungi/mould contamination of the fishery products during storage (Kolakowska, 2002). But subsequent increase during storage period may be due to storage of the dry fishes at ambient temperature or may be due to absorption of moisture from atmosphere which accelerates the mould growth.

#### 5.10. Organoleptic changes during storage:

The results of organoleptic evaluation during storage of dry Bombay duck and ribbon fish of different samples ( $T_1$ , C, and  $T_2$ ) shows that there was a decline in overall quality characteristics, namely colour and appearance, odour, texture, fish flavour intensity and overall acceptability during storage period of 120 days. In case  $T_1$  samples, overall acceptability of dry Bombay duck and ribbon fishes were reduced upto  $6.42 \pm 0.50$  and  $6.16 \pm 0.79$  respectively (Table. 30) (Fig.27) whereas for  $T_2$  sample, the acceptability of the dry fishes in same order of both the species were reduced upto  $6.08 \pm 0.17$  and  $6.17 \pm 0.79$  (Table. 31) (Fig.28). However, the overall acceptability for control sample of both the species scored less value during storage study (Table. 29) (Fig.26). The better quality of treated samples ( $T_1$  and  $T_2$ ) with preservatives, than the control samples (C) was depicted by its higher scores throughout the storage period. At the end of storage period of 120 days, the appearance of control sample was dull, colour brownish yellow, texture less firm, odour considerably off and the panellist rated the dried fishes as 'Dislike Moderately' ('3' points). The experimental findings (Table. 29-31) indicate that the dry salting is better than the brining and use of preservatives enhances quality as well as the shelf life of the product which shows similarity with the findings of Khuntia *et al.*, (1990).



*Chapter - 6*

**Summary and Conclusion**

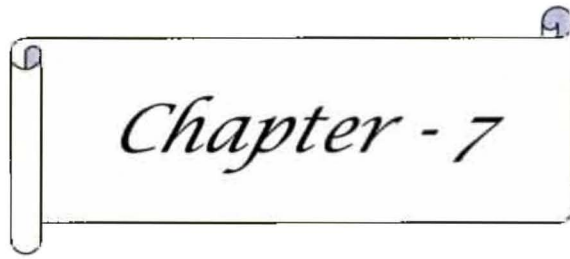
The study includes to analyse the quality of dry fishes (*Harpodon nehereus* and *Lepturacanthus savala*) produced at Junput and Jalda khoti and also storage study of these dry fishes treated with different preservatives as well as end product utilization of these dried products. A brief summary of the present work is outlined below:

1. Drying activity follows open sun drying of fishes in the two different coastal khotis (i.e. Jalda and Junput Khoti) of West Bengal. However, there is some difference in the method practiced for drying between the two khotis, i.e., in case of Junput khoti, the fishes are dip treated in salt and chemical solution prior to drying. On the other hand, in Jalda khoti, the fishes are dried without any treatment. Beside this, other activities followed in these khotis are almost same (Table. 5).
2. The species collected from Junput khoti were better in terms of microbial and biochemical qualities which may be due to salt and chemical treatment given to the fish prior to drying. In case of Jalda khoti, no such treatments were given which makes it more susceptible to spoilage. However the moisture content of Junput dry fishes were more, which in turn results higher fungal /mould growth in both the species.
3. Three different samples of both Bombay duck and ribbon fish were treated separately with saturated brine (C), dry salt with 0.25 % potassium sorbate (T<sub>1</sub>) and saturated brine with 0.3% sodium benzoate (T<sub>2</sub>). The samples were kept for drying following the similar condition as existing in the fields and packed in polythene (LDPE) packets for 120 days storage study.
4. The results of biochemical parameters revealed that during storage period, TVBN, TMA and PV content of T<sub>2</sub> was better than C and T<sub>1</sub> both in Bombay duck and Ribbon fish samples. Whereas in Bombay duck, TBA value of C was better than T<sub>1</sub> and T<sub>2</sub> and in case of Ribbon fish, T<sub>1</sub> was better than C and T<sub>2</sub> samples.
5. The results of microbiological parameters revealed that the quality of the dry fishes of both the samples (T<sub>1</sub> and T<sub>2</sub>), treated with antimicrobial preservatives, was of good quality and remains acceptable for longer duration than the control one. TPC values of both Bombay duck and ribbon fishes of T<sub>1</sub> sample were significantly ( $p < 0.05$ ) lower than T<sub>2</sub> and C sample (Table. 25-

26). For dry Bombay duck, fungal count of T<sub>2</sub> sample shows significantly ( $p < 0.05$ ) lower value than T<sub>1</sub> and C sample but in case of ribbon fish, T<sub>1</sub> shows significant ( $p < 0.05$ ) lower value than T<sub>2</sub> and C sample. The results showed that, the values of dry Bombay duck and ribbon fish of T<sub>1</sub> and T<sub>2</sub> samples reached to unacceptable limit after a period of 90 days (Table. 27-28) during storage period.

6. The sensory analysis was carried out using 9 point hedonic scale. The results revealed that both T<sub>1</sub> and T<sub>2</sub> were within the acceptable limit till the end of storage study of both Bombay duck and Ribbon fish samples. Whereas, in case of control (C) samples, it became slightly disliked at the end of the storage period (Table. 29).

Dry fish is an important source of animal protein particularly for the poorer section of the society. However, in India the production process of dry fish is unhygienic which results in inferior quality of the final product. The idea of improving the quality of dry fish by using chemical preservatives was to give extended shelf life to the dry fish which enables the entrepreneurs to make it available to the north-eastern states of the country with better quality. From the present study, it may be concluded that use of permissible food grade chemicals can increase the shelf life of the dry fish to an extended period which may be beneficial for both producers and consumers.



*Chapter - 7*

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*Chapter - 8*

**ANNEXURE**

**APPENDIX - I**

**I. Organoleptic evaluation of salted and dried fish based on hedonic scale  
(ANNEXURE-I)**

<b>SCORE</b>	<b>9</b>	<b>8</b>	<b>7</b>	<b>6</b>	<b>5</b>	<b>4</b>	<b>3</b>	<b>2</b>	<b>1</b>
<b>ATTRIBUTE</b>									
<b>Colour and Appearance</b>	Extremely Bright	Very much Bright	Bright	Moderately Bright	Slightly Bright	Slightly Dull	Moderately Dull	Dull	Extremely Dull
<b>Odour</b>	Extremely Desirable	Very much Desirable	Desirable	Not Detectable	Not Easily Detectable off Flavor	Slightly Detectable off Flavor	Moderately Detectable off Flavor	Detectable of Flavor	Putrid Flavor
<b>Texture</b>	Extremely Desirable	Very much Desirable	Desirable	Not Detectable	Not Easily Detectable Poor Texture	Slightly Detectable Poor Texture	Moderately Detectable Poor Texture	Detectable Poor Texture	Poor Texture
<b>Fish Flavor Intensity</b>	Very much Extremely	Like Extremely	Like	Like Moderately	Like Slightly	Dislike Slightly	Dislike Moderately	Dislike	Extremely Dislike

**Score sheet for evaluation of salted and dried fish  
based on 9 point hedonic scale (ANNEXTURE: II)**

<b>Sample</b>	<b>Colour &amp; Appearance</b>	<b>Odour</b>	<b>Texture</b>	<b>Fish Flavor Intensity</b>	<b>Overall Acceptability</b>
<b>CB</b>					
<b>CR</b>					
<b>T<sub>1</sub>B</b>					
<b>T<sub>1</sub>R</b>					
<b>T<sub>2</sub>B</b>					
<b>T<sub>2</sub>R</b>					

**Remarks:**

**Signature of the panellist**

**Date:**