Chapter 1

Introduction
Experience over many centuries has established the fact that groups of people who obtain their meat supplies from the oceans, rivers or lakes are well nourished (Geiger & Borgstrom, 1962). The tall and healthy type of individual may be found either along the coastline where large quantities of fish are consumed or in the cattle-raising lands where the intake of animal protein is high. Manipur, a small state in the north east corner of India, which is blessed with several freshwater resources is no exception. Majority of the people of the state take fish as the only source of animal protein in their diet (Hora, 1921).

Well-organized medical surveys have demonstrated the adequacy of fish as the principal source of dietary protein. Its composition is comparable to those of higher vertebrates in quality, but differs in containing less collagen and more extractives (CSIR, 1962). It is rich in lysine and methionine. Deficiency of any one essential amino acid (EAA) in diet is a limiting factor in growth and other metabolic activities. Thus, addition of fish in
human diet serves as an excellent source of EAA (Uddin & Rajbansi, 1984). Apart from this, fish is rich in unsaturated fatty acid and minerals.

The rapid growth in human population and depletion of available water resources for other activities resulted in the inability to supply the amount of fish required. The actual fish production of the state in 1991 was only 9,950 metric tonnes (Department of Fisheries, 1992), while the population was 18.27 lakhs (Census of India, 1991). In order to meet the additional requirement, large quantities of iced and smoked fishes are obtained from neighbouring states.

A monthly market survey for one year revealed that approximately 120 metric tonnes of iced fishes are brought annually from states like Assam, Andhra Pradesh, Bihar, West Bengal, U.P. etc. Maximum amounts of fishes are brought in the months starting from August of the year to May of the following year. The species of iced fishes which are sold in the Imphal market (the main fish market of Manipur) are Wallago attu, Labeo rohita, L. gonius, Hilsa ilisha, Notopterus chitala and Aorichthys aor in the decreasing order of quantity sold.

Smoked fishes brought from the state of Assam are: Channa sp, Anabas testudineus, W. attu, Lepidocephalus sp. which amounts to about 60 metric tonnes per annum. Out of
these, smoked _Channa_ are brought in higher quantity. In addition, about 10 metric tonnes of smoked fishes of _Garra_, _Tor_ and _Puntius_ are brought from Myanmar and sold at Moreh, boarder town of Manipur.

Although the required amount of fish may be procured from other states, the basic concern in the status of the fish in relation to human nutrition and hygiene when reach the consumers. It generally takes 1 to 2 days for iced fishes to reach Calcutta from the site of catch (Govindan, 1985). It takes another 4 to 5 days for them to reach different marketing centres of north eastern states.

The fishes that reach Imphal are packed with ice in the ratio of 2-3:1 by volume in bamboo or plywood boxes, lined with leaves and gunny bags. For insulation, saw dust or paddy husk are also incorporated in the packing. After reaching the market, the fishes are generally transferred to new plywood boxes and fresh ice is added.

Ice storage retard spoilage of fishes by suppressing the activity of bacteria and enzymatic and non enzymatic degradation. Exposure of fresh fishes to air reduces its shelf life. Thus, shelf life of iced fishes depends on the time lapsed between fishing and icing. Icing causes loss of nutrient rich tissue water as drip, leaching of flavour components, loss of nutritionally desirable minerals and water soluble protein (Holston & Slavin, 1965; Rao & Bhagirathi, 1985).

Smoking has been practiced by Manipuris since time immemorial as technique for preservation of fish and to obtain smoky flavour. A brief account on the technique was reported by Singh _et al_ (1990). Smoking not only improves the organoleptic qualities of the fish, but also extends the shelf life by delaying the onset of fungal attack. Smoke deposited on the fish in the process has preservative effect since it contains formaldehyde, phenols and
other substances evolved from smoke (Stansby, 1963). Hot smoking may result in the loss of the nutritive quality of protein, especially EAA, such as lysine, tryptophan, cystine and histidine (Bhuiyan et al., 1986a & b, Boge, 1960). Cold smoking or a temperature not higher than 42°C does not sufficiently alter amino acid composition of fish protein (Carpenter, 1960).

Determination of digestible protein gives the value of the nutritionally available protein. The simplest method is to evaluate nitrogen (N) retained in an animal, i.e., the difference between N intake and N excreted. Evaluation of protein and amino acid through growth, however, a rigorous method, integrating most of the functions of proteins into one measurement. Estimation of \textit{in vitro} protein digestibility (PD) using enzyme is another acceptable and simple methods for evaluating protein quality and its availability in foods (Hsu et al., 1977).

Muscle of living fish is generally considered sterile. Skin, gill and visceral organs of freshly caught fishes, on the other hand, have been found to have many bacteria. Following death, the microorganisms grow and move into various tissues depending on their types and temperature at which the fish are held (Graikoski, 1973). Some bacteria even propagate at refrigeration temperatures.

Bacteria secrete digestive juices and enzymes that break down the tissues they attack and spoil the fish in turn. Certain moulds also contribute to spoilage. Determination of total counts of bacteria gives useful information regarding microbial quality and characteristic spoilage patterns that safeguard the consumers (Silliker, 1963).
Low total count, on the other hand, is no assurance of freedom from pathogens. Pathogenic organisms may not contribute much to spoilage as others do. *Salmonella* has been detected in meat with extremely low count of bacteria and with no organism of faecal origin (Hobbs & Wilson, 1959). Hot smoked eel caught from contaminated waters caused outbreak of *Salmonella* poisoning in Holland (Van der Brock, 1948). There are also reports on the contamination of *Staphylococcus aureus* in fishery products. It was isolated from palms and throats of workers in processing factories of Cochin (Sanjeev & Iyer, 1988, Iyer & Shrivastava, 1988). It could cause gastroenteritis as it secretes an endotoxin (Jay, 1978) which caused diarrhoea, vomiting general malaise and prostration etc. (Iyer, 1979a). Presence of coliforms, *E. coli*, faecal *Streptococci* etc give an indication of faecal contamination and of enteric pathogens (Shewan, 1962; Frazier & Westhoff, 1983).

Bacterial flora of fish show considerable variation in relation to the environmental condition in which the fish live (Shewan, 1971; Surendran & Gopakumar, 1983; Fredrick & Thomas, 1985). Composition of the macroflora and their counts is greatly influenced by processing (Pivovarov *et al*, 1988) and marketing system (Rao & Bhagirathi, 1985). Bacterial load in gut is a function of food ingestion and that of skin and gill, of environment, season, handling and processing (Shewan, 1961). Food requirements of man and microbes are much the same, which is why fish spoils so rapidly once they have been contaminated by bacteria in sufficient numbers (Iyer, 1979b).

Most of the bacteria of fresh fishes are mesophiles (Joseph *et al*, 1988) and that of iced, are psychrophiles (Araujo *et al*, 1989). Spoilage of smoked fishes are mostly related to nonspore forming rod which survive the processing or those introduced during handling of the product (Graikoski, 1973).
Fig. 1. Section of Imphal Fish Market for Iced Fishes

Fig. 2. Mode of sale for Iced Fishes
Section of Imphal Fish Market for Smoked Fishes
Mycotoxicoses in human and livestock are also of great concern (Rodricks & Pohland, 1981). Inadvertent exposure to toxic fungi and fungal metabolites cannot be ruled out unless proper care is taken.

With the advancement of science and technology and the growing awareness among the people, more emphasis have now been given on the quality rather than the quantity of product. The necessity of examining a detailed biochemical, nutritional and microbiological quality of all the fish samples sold at the Imphal market of Manipur with special reference to the health and hygiene of consumers has been felt. Thus, common species of fresh, iced and smoked fishes were selected and investigations were made as follows:

**Fish Samples Examined**

**Fresh:** *Anabas testudineus, Clarias batrachus, Labeo rohita, Lepidocephalus guntea, Monopterus albus* and *Wallago attu.*

**Iced:** *Aorichthys aor, Hilsa ilisha, Labeo gonius, L. rohita, Notopterus chitala* and *Wallago attu.*

**Smoked:** *Anabas testudineus, Channa punctatus, Clarias batrachus, Puntius jayarami, Tor putitora* and *Wallago attu.*

The work was carried out with the following objectives:

A. Determination of Biochemical Composition, examination of Spoilage and Sensory/Organoleptic qualities:

i) determination of proximate composition;

ii) determination of total volatile base N, thiobarbituric acid number, free fatty acid value, non protein N and pH.

iii) evaluation of sensory quality and reconstitution properties.
B. Judgment of Nutritional Quality:
   i) growth of experimental rats fed with fish as source of animal protein,
      protein efficiency ratio, digestibility and biological value of protein
   ii) protein digestibility in vitro
   iii) estimation of alpha-amino N liberated during enzyme action

C. Assessment of Microbiological Quality and detection of Pathogenic Microorganisms:
   i) total bacterial and fungal counts
   ii) detection of pathogenic bacteria and counts of coliforms

D. Identification of Microflora Associated with Fish:
   i) bacterial flora and their percentage composition
   ii) fungal flora and their percentage composition

The results are presented in the following chapters and discussed keeping in view of the data already available. Suggestions for improvement of quality have also been made.