Chapter II

Biochemical Composition, Spoilage
& Sensory Evaluation
BIOCHEMICAL COMPOSITION

The primary factors which determine the value of a fish with reference to its edible and keeping quality and suitability for various processing method is its biochemical composition. The composition in fishes is different between species and in individuals of the same species depending on their distribution, age, sex, maturity etc., (Jafri, 1968; Bano, 1977).

Fish is a protein rich food and thus forms an important part in the human diet. It is known for its easy digestibility and high nutritive value. Animal proteins, especially fish proteins are rich in two essential amino acids (EAA), viz.; lysine and methionine when compared with cereal proteins. Deficiency of any one EAA is a limiting factor as other EAA present in the diet become inactive. Thus, addition of fish in human diet serves as excellent source of EAA (Uddin and Rajbansi, 1984).
Animal protein constitutes one third of the total requirement of protein in the daily diet of human beings. Fish provides 80 percent of the animal protein consumed in our country. Fish protein is considered to be of high quality since it is comparable to those of meat, milk and eggs. The edible portions of freshwater fish contain 14-25 per cent protein, the composition of which is much similar to those of higher vertebrates in quality, but differs in containing less collagen and more extractives (CSIR, 1962).

Lipid content of fishes varies from 6 to 20 per cent (Guha, 1962). Freshwater fish is recommended in the diet as it contain low lipid and high protein. Fish lipid, in general consists of primary triglycerides, esters of fatty acids, less amount of free fatty acids, sterols, vitamins and hydrocarbons. A diet of fish can reduce the chance of heart attack in man since fish oil inhibits production of a protein called PDGFc. This protein stimulates growth of vascular smooth muscle cells which penetrate the wall of blood vessels and cause constrictions in the flow of blood (Khan, 1988). Under these conditions, a blood clot can easily block the vessel and cause heart attack. Although fish is very unlikely to be the only source of an essential mineral in the diet, it provides a well-balanced supply of minerals. Fish is rich in calcium, phosphorous and fluorine, which are ideal for teeth and building of bone. Mineral content in fish muscle varies from 1 to 2 per cent (CSIR, 1962).

Water content in fish is high, hence spoils easily by the action of intrinsic enzymes and microorganisms. Water content shows inverse relationship with that of fat in the fatty fishes and with that of protein in the non-fatty fishes (Bano, 1977).

Storage/Processing Quality - It has been reported that ice storage causes decrease in total protein and non protein nitrogen (Joseph et al., 1980; Garg & Stephan,
1982, Reddy & Srikar, 1991). Hot smoking of fish results in loss of protein along with weight loss (Bhuiyan et al., 1968a). Smoking or dehydration at a temperature not higher than 42°C does not significantly affect amino acid composition of fish protein. Severe heat treatment during processing may result in the unavailability of lysine units in proteins (Carpenter, 1960). Weight loss has also been reported (Shewan, 1949a; Bhuiyan et al., 1986b) in the process of smoking.

The qualities of the finished product largely depend on the quality of raw material and processing method used. 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 wood smoke. (Stansby, 1963). During smoking, the action of heat brings about the drying effect. The rise in temperature in the process may volatalize some objectionable compounds present in the fish (Connell, 1975).

**Spoilage** - Fish is highly perishable and spoilage sets in soon after catch. Spoilage is brought about by enzymatic, bacterial and non enzymatic degradations; one of the factors that alters the autolytic and bacterial decomposition of the flesh is rigor mortis, i.e. the death and stiffening of the muscle. In normal conditions, it lasts from 1 to 7 hours after death (Amlacher, 1961). Soon after death, anaerobic glycolysis start, resulting in the accumulation of lactic acid. As a result, pH falls down to 5.4. Myofibrillar protein actin and myosin combine to form actomyosin resulting in stiffening of muscle in rigor mortis. Since the antioxidant tocophenol (Vitamin E) supply is cut off, the lipid in the tissue undergoes rapid oxidation by atmospheric oxygen and thus oxidative rancidity sets in (Jasmine, 1985).
Perigreen et al. (1987) suggested that thiobarbituric acid (TBA) value and free fatty acid (FFA) value increased with strong rancid odour as a result of lipid hydrolysis during spoilage of fish. Oxidation of fish lipid is more rapid than of other meats. Some lipolytic bacteria produce lipase which plays a vital role in degrading the fat in food resulting in quick deterioration. Measurement of simple fat analytical techniques, viz., FFA, peroxide value (POV) and TBA values are important in quality control of fish (Obanu, 1987).

Total N, non protein N and total volatile base nitrogen (TVBN) value showed a decreasing trend during iced storage (Devadasan et al., 1978; Garg & Stephan, 1982). Drip loss and leaching are common phenomena in iced storage. pH of fresh flesh influence rigor mortis and growth of bacteria. After death, pH is lowered due to the conversion of glycogen to lactic acid, which in turn rupture lysosomal membrane. By this, tissue components are degraded into amino acids, peptones, nucleotides etc. As a result, pH gradually increases up to 7.0. The degraded compounds offer an ideal medium for microbial growth.

Measurement of spoilage in fish depends upon the complex series of changes in the constituents of flesh brought about by autolytic enzymes and putrefactive micro-organisms. During spoilage, non protein N (NPN) and TVBN increases due to the denaturing of nitrogen compounds. FFA and volatile acids increase due to oxidative rancidity and enzyme lipolysis (Jasmine, 1985). In smoked fishes, increase in TVBN has no significant effect on the organoleptic qualities of the product.

**Sensory Test** - The freshness of fish largely depends on the temperature at which it has been kept and the time lapsed after it was caught. The higher temperature during handling and distribution cause spoilage of lipid. Freshness is usually judged in the trade
entirely by appearance, odour and texture upon the senses. These factors are known as sensory or organoleptic.

Shelf life of iced fishes depends on the time lapsed between fishing and icing. The life is decreased when iced fishes are exposed for a long time to direct sunlight. A delay in chilling for a period after death which approximately equals to the onset and duration of rigor mortis does not cause any noticeable decrease in subsequent self life in ice (Nair, 1985a). In case of fresh fish, exposure to air after harvesting reduces shelf life.

Report on the biochemical composition of fishes of Manipur are scanty except for a few reports on some fresh fishes by Sarojnalini and Vishwanath (1988a,); Vishwanath and Sarojnalini (1988), on smoked fishes by Singh et al. (1990); Lilabati et al. (1993) and on iced fishes by Vishwanath and Lilabati (1995).

In the present work, biochemical composition, Spoilage and sensory condition of six fresh fishes (Clarias batrachus, Wallago attu, Labeo rohita, Anabas testudineus, Lepedocephalus guntea and Monopterus albus), six iced fishes (Wallago attu, Labeo rohita, Labeo gonius, Aorichthys aor, Notopterus chitala and Hilsa ilisha) and six smoked fishes (Clarias batrachus, Wallago attu, Anabas testudineus, Channa punctatus, Tor putitora and Puntius jayarami) commonly available in Manipur were studied.
MATERIALS AND METHODS

Materials - Fish samples taken for study were:

**Fresh Fishes:** *Clarias batrachus* (12.10-14.00 cm, 21.2-32.8 gm); *Wallago attu* (66-68 cm, 1300-1400 gm); *Labio rohita* (40-45 cm, 800-1200 gm); *Anabas testudineus* (8.1-9.2 cm, 20.0-31.2 gm); *Lepidophalus guntea* (5.5-5.9 cm, 1.5-4.5 gm); *Monopterus albus* (57.0-60.2 cm, 15.65-20.2 gm).

**Iced Fishes:** *Wallago attu* (50-72 cm, 1-1.6 kg); *Labio rohita* (40-47 cm, 1.0-1.4 kg); *Labio gonius* (29-35 cm, 0.7-1.2 kg); *Aorichthys aor* (60-76 cm, 1.7-3.0 kg); *Notopterus chitala* (60-76 cm, 1.5-2.0 kg); *Hilsa ilisha* (28-32 cm, 0.8-1.3 kg).

**Smoked Fishes:** *Clarias batrachus* (10.5-13.6 cm, 12.6-18.27 gm); *Anabas testudineus* (6.0-7.5 cm, 2.97-7.19 gm); *Channa punctatus* (8.4-10.6 cm, 3.34-10.17 gm); *Wallago attu* (19.2-24.0 cm, 11.03-24.4 gm); *Tor putitora* (15-23 cm, 20.3-45.0 gm); *Puntius jayarami* (7.0-11.0 cm, 2.5-4.15 gm)

Three fishes for every species were selected randomly each from different vendors. Fishes were collected aseptically in polythene bags and brought to the laboratory. Such samplings were done weekly for three months.

Methods

**PROXIMATE COMPOSITION**

50 g. muscle was sampled randomly from different parts of the body and then used for various analyses. **Moisture** content was determined by hot air oven method (Hart & Fisher, 1971), by drying at 105±1°C in a hot air oven till constant weight. The content was
expressed as percentage of the sample taken. For determination of Crude Protein content, N content in samples were determined by Kjeldahl digestion followed by spectrophotometric estimation after nesslerization of the digest (Lang, 1958). The value of protein was obtained by multiplying N value with 6.25 in case of animal samples and with 5.7 for plant samples (Osborn & Voogt, 1978). Lipid content was determined by extraction with chloroform: methanol following the method of Folch et al., (1957). For Ash content, moisture free sample was ignited at 550°C for 2-3 hours as described by ISI (1982).

**SPOILAGE STUDIES**

**Non protein N** (NPN) was determined by the difference between total N and pure protein N (AOAC, 1975). The sample was treated with trichloroacetic acid (TCA) so as to precipitate protein. N content in the precipitate was determined. The difference between the N content of the sample and that of the precipitate gives the value of NPN.

**Total Volatile N** (TVN) was determined by microdiffusion technique using a conway apparatus (Morris, 1959). Volatile bases are allowed to diffuse into a known concentration of dilute HCl. The change in the normality of the acid overnight was determined by titrating against an alkali, viz., NaOH. The value was expressed in mg per gram sample.

**Thiobarbituric Acid** (TBA) number was estimated by the method of Sinhuber & Yu (1958). Oxidised lipid reacts with 2-thiobarbituric acid to form a red coloured compound melonaldehyde. Colour was read spectrophotometrically. The extrapolated value was multiplied by a constant factor 7.5 to give TBA no. The value was expressed as mg melonaldehyde per kg of sample.
To determine **Free Fatty Acid** (FFA), lipid was treated with neutral alcohol, which was titrated against NaOH using phenolphthalein as indicator (Morris, 1959).

pH of the samples was determined by the method of Valsan (1975). 1.0 gm of fish muscle was homogenised with 10.0 of distilled water and pH was recorded using a pH meter.

**SENSORY EVALUATION**

The parameters taken for sensory evaluation for smoked fishes were: colour, texture and odour and those for fresh and iced fishes were: general appearance of eyes, gill, slime, odour, textures etc. They were judged by a panel of six judges that includes one student, four research scholars and a teacher of the Department. Smoky odour was recorded in a three-point scale i.e. '+' for pleasant, '+' for moderate and '+' for poor smoky odours. For iced and fresh fishes, the score was given on ten point hedonic scales [poor (1-2), fair (3-5), good (6-8), excellent (9-10)]. General appearances of the fishes were recorded based on simple eye estimation and texture by applying pressure to the fish samples by fingertips. Odour was adjudged by smelling.

**RECONSTITUTION PROPERTIES**

In smoked fishes it was studied based on the method of Valsan (1975). 10 gm of muscle sample was taken and soaked in 50 ml of distilled water for 3½ hours. Excess of water was drained and reconstitution was expressed as percentage of water imbibed by the sample.
Fig. 1. Fresh *Wallago attu*

Fig. 2. Fresh *Labeo rohita*
Fig. 1. Fresh *Clarias batrachus*

Fig. 2. Fresh *Anabas testudineus*
Fig. 1. Fresh *Lepidocephalus guntea*

Fig. 2. Fresh *Monopterus albus*
Fig. 1. Iced _Labeo rohita_

Fig. 2. Iced _Labeo gonius_
Fig. 1. Iced *Wallago attu*

Fig. 2. Iced *Notopterus chitala*
Fig. 1. Iced *Hilsa ilisha*

Fig. 2. Iced *Acrothys aor*
Fig. 1. Smoked *Anabas testudineus*

Fig. 2. Smoked *Channa punctatus*
Smoked *Wallago attu*
Fig. 1. Smoked *Puntius jayarami*

Fig. 2. Smoked *Tor putitora*
RESULTS

BIOCHEMICAL COMPOSITION

Fresh Fishes

Table I (Fig. 2.1) shows the biochemical composition of six fresh fishes i.e. *C. batrachus*, *W. attu*, *L. rohita*, *L. guntea*, *M. albus* and *A. testudineus*.

**Moisture** - Moisture content ranged from 75.00 to 79.43 per cent. Highest was in *W. attu* and lowest in *L. guntea*. **Crude protein** - Value is expressed in per cent on dry weight basis. Out of six fresh fishes, *W attu* had the highest protein value of 81.28, followed by *C. batrachus* (81.03), *L. rohita* (79.56), *M. albus* (78.96), *A. testudineus* (76.02) and *L. guntea* (70.00) in decreasing order. **Lipid** - Lipid content of the fishes varied from 5.74 to 12.26 per cent on dry weight basis. Highest was observed in *A. testudineus* (12.26) and next by *C. batrachus* (11.00) and *L. guntea* (11.00), and lowest by *M. albus* (5.74). **Ash** - It was within the range of 4.00 to 13.00 per cent on dry wt. basis. In decreasing order the values of ash were 13.00, 7.33, 7.00, 5.98, 5.02, 4.00 were observed in *L. guntea*, *A. testudineus*, *M. albus*, *W. attu*, *L. rohita*, *C. batrachus* respectively.

**Non protein nitrogen** (NPN) - The value are in per cent on dry weight basis. Highest value of NPN was found in *L. rohita*...
In decreasing order the values of, \( W. \text{attu}, L. \text{guntea}, M. \text{albus}, C. \text{batrachus} \) and \( A. \text{testudineus} \) were 2.53, 2.28, 1.96, 1.80 and 1.61 respectively.

**Total volatile base nitrogen (TVBN)** - TVBN value of the fresh fishes were low. It was between 9.00 mg per cent in \( L. \text{guntea} \) and 11.00 mg per cent in \( W. \text{attu} \).

**pH** - pH values were slightly acidic. It was within the range of 6.58 to 6.90. Highest was recorded in \( M. \text{albus} \) and lowest in \( L. \text{rohita} \).

### Iced Fishes

Biochemical composition of six iced fishes i.e. \( w.\text{attu}, L. \text{rohita}, L. \text{gonius}, A. \text{aor}, N. \text{chitala}, H. \text{ilisha} \) are in table 2 (Fig. 2.2).

**Moisture** - Moisture content of the iced fishes were between 78.00 and 80.47 per cent. The highest was of \( W. \text{attu} \) and the lowest, of \( L. \text{gonius} \). **Crude protein** - Crude protein content (per cent on dry wt. basis) was moderately high, which ranged from 70.38 to 84.59. \( L. \text{gonius} \) had the highest value, 84.59. Their values in decreasing order were of \( w. \text{attu}, 79.26; H. \text{ilisha}, 77.59 \) and \( A. \text{aor}, 70.38 \).

**Lipid** - Among the six iced fishes lipid content (per cent on dry wt. basis) was highest in \( A. \text{aor} \).
aor (20.62), followed by *H. ilisha* (14.02); *W. attu* (7.83), *L. rohita* (7.46), *N. chitala* (6.51)
and lowest in *L. gonius* (5.91). **Ash** - It was slightly similar in all the fishes. *N. chitala* had the highest value, 4.01 and the lowest, in *L. rohita* 3.30% in dry wt. basis.

**Non protein nitrogen (NPN)** - Value is expressed in per cent dry wt. basis. Among the six iced fishes analysed, *H. ilisha* had the highest NPN value (2.46) followed by *W. attu* (2.41), *A. aor* (2.36), *L. gonius* (2.22), *N. chitala* (2.14) and *L. rohita* (1.58) in decreasing order. **Total volatile base nitrogen (TVBN)** - TVBV value of 14.00 and 12.00 mg per cent were observed. *A. aor* and *N. chitala* had similar value of 14.00 mg per cent each and *W. attu*, *L. rohita*, *L. gonius* and *H. ilisha*, had the similar value of 12.00 mg per cent each. **Free fatty acid (FFA)** - The highest value of FFA (per cent oleic acid) was recorded in *N. chitala* (6.35) and the lowest, in *L. gonius* (2.50). **Thiobarbituric acid (TBA)** - TBA value (mg malonaldehyde/1000 gm) of six iced fishes were within 0.49-0.75. The values in *W. attu*, *N.chitala*, *L. gonius*, *A. aor*, *L. rohita* and *H. ilisha* were in decreasing order: 0.75, 0.62, 0.59, 0.56, 0.51 and 0.49 respectively. **pH** - In all the samples were the values were of slightly acidic nature. It was in the range from 6.40 to 6.90. Highest was found in *H. ilisha* and lowest in *N. chitala*.

**Smoked Fishes**

Results of analysis of six smoked fishes i.e. *C. batrachus*, *W. attu*, *A. testudineus*, *C. punctatus*, *T. putitora* and *P. jayarami* are presented in table 3 (Fig. 2.3).

Out of the six smoked fishes studied *C. batrachus* was produce of Manipur while the other five were of outside Manipur origin.
Moisture - Moisture content of six smoked fishes in decreasing order (in per cent) were, *C. batrachus*, 47.58; *P. jayarami*, 15.00; *T. putitora*, 6.00; *W. attu*, 5.80; *C. punctatus*, 5.71 and the lowest, in *A. testudineus*, 3.65.

Crude protein - Values were expressed in per cent dry weight basis (DWB). Highest was of *P. jayarami*, 83.40, followed by *W. attu*, 79.73; *C. punctatus*, 76.37; *T. putitora*, 76.04; *A. testudineus*, 73.74 and the lowest of *C. batrachus*, 71.44. Total lipid - Lipid content (per cent DWB) of the fishes varied from 3.60 to 10.73. The highest was observed in *T. putitora* and the lowest, in *C. punctatus*. Ash - Ash content of the fishes were within the range of 4.00 to 9.41 (per cent DWB). Highest was recorded in *C. punctatus* and lowest in *C. batrachus*.

Non protein nitrogen (NPN) - NPN varied from 0.83 to 4.85 (per cent DWB). The values of 4.85, 3.90, 3.04, 2.51, 1.92 and 0.83 were observed in *T. putitora, P. jayarami, W. attu, A. testudineus, C. punctatus* and *C. batrachus* respectively in decreasing order.

Total volatile base nitrogen (TVBN) - The values were expressed in mg per cent. It was within the range of 24.00 and 39.00. The highest value was measured of *W. attu* and
the lowest, of *A. testudineus*. **Free fatty acid (FFA)** - The values were within the range of 4.40 and 8.65 (% olic acid). The highest was of *W. attu* and the lowest, of *P. jayarami*. **pH** - pH value of six fishes were slightly acidic. It was within the range of 6.30 and 6.42. In three fishes, i.e. *W. attu*, *C. punctatus* and *P. jayarami*, the value was 6.3; in the two fishes i.e. *A. testudineus* and *T. putitora* the value was 6.4. The highest value of 6.42 was observed in *C. batrachus*.

**SENSORY EVALUATION**

**Fresh Fishes**

Organoleptic quality of six fresh fishes viz., *C. batrachus*, *W. attu*, *L. rohita*, *L. guntea*, *M. albus* and *A. testudineus* were excellent as they scored 9-10 (Table 5). Eyes and gills were in very good condition. They had firm texture and fresh fish odour.

**Iced Fishes**

Out of the six iced fishes studied, organoleptic quality of *A. aor* was adjudged good (6-8) and that of other fishes i.e. *W. attu*, *L. rohita*, *L. gonius*, *N. chitata* and *H. ilisha* was adjudged fair (3-5) (Table 4). Some specimens of *W. attu* and *H. ilisha* had slightly rancid odour. Most of the iced fishes had soft texture and neutral odour.

**RECONSTITUTIONAL AND SENSORY EVALUATION OF SMOKED FISHES**

Reconstitution property were highest in *C. batrachus* was highest (194.00). The values in decreasing were of *P. jayarami* (142.00), *C. punctatus* (123.00), *A. testudineus* (117.67), *W. attu* (97.67) and *T. putitora* (90.00). *C. batrachus* had soft texture. *P. jayarami*
was slightly soft while the remaining four fishes had very hard texture. *P. jayarami* had light yellow colour, *T. putitora* and *W. attu* were yellowish black and remaining three fishes were black in colour. *C. batrachus*, *P. jayarami* and *T. putitora* had pleasant smoky odour whereas *A. testudineus*, *W. attu* and *C. punctatus* had medium smoky odour.
### TABLE 1. Biochemical Composition of Fresh Fishes

<table>
<thead>
<tr>
<th>Composition</th>
<th>C. batrachus</th>
<th>W. attu</th>
<th>L. rohita</th>
<th>L. guntea</th>
<th>M. albus</th>
<th>A. testudineus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>79.01±0.28</td>
<td>79.43±0.21</td>
<td>78.70±0.48</td>
<td>75.00±0.85</td>
<td>77.00±0.71</td>
<td>77.90±0.20</td>
</tr>
<tr>
<td>Crude protein (% DWB)</td>
<td>81.03±0.42</td>
<td>81.28±0.26</td>
<td>80.58±0.32</td>
<td>70.00±0.72</td>
<td>78.96±0.80</td>
<td>76.02±0.65</td>
</tr>
<tr>
<td>Total lipid (%) DWB</td>
<td>11.00±0.01</td>
<td>9.63±0.07</td>
<td>9.72±0.06</td>
<td>11.00±0.05</td>
<td>5.74±0.02</td>
<td>12.26±0.01</td>
</tr>
<tr>
<td>Ash (% DWB)</td>
<td>4.00±0.00</td>
<td>5.98±0.10</td>
<td>5.02±0.01</td>
<td>13.00±0.50</td>
<td>7.00±0.06</td>
<td>7.33±0.01</td>
</tr>
<tr>
<td>NPN (%) DWB</td>
<td>1.80±0.01</td>
<td>2.53±0.00</td>
<td>2.54±0.02</td>
<td>2.28±0.01</td>
<td>1.96±0.01</td>
<td>1.61±0.05</td>
</tr>
<tr>
<td>TVBN (mg%)</td>
<td>10.50±1.00</td>
<td>11.00±1.00</td>
<td>10.00±0.00</td>
<td>9.00±1.00</td>
<td>10.00±0.00</td>
<td>9.10±2.00</td>
</tr>
<tr>
<td>pH</td>
<td>6.84±0.00</td>
<td>6.75±0.00</td>
<td>6.58±0.10</td>
<td>6.80±0.01</td>
<td>6.90±0.01</td>
<td>6.80±0.04</td>
</tr>
</tbody>
</table>

Note: Results are mean ± S.D. of 8 samplings.

DWB = Dry weight basis
NPN = Non protein nitrogen
TVBN = Total volatile base nitrogen
<table>
<thead>
<tr>
<th>Composition</th>
<th><em>W. attu</em></th>
<th><em>L. rohita</em></th>
<th><em>L. gonius</em></th>
<th><em>A. aor</em></th>
<th><em>N. chitala</em></th>
<th><em>H. ilisha</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>80.47±0.58</td>
<td>80.30±0.41</td>
<td>78.00±0.42</td>
<td>78.66±0.52</td>
<td>78.80±0.37</td>
<td>79.38±0.62</td>
</tr>
<tr>
<td>Crude protein (% DWB)</td>
<td>79.26±0.39</td>
<td>80.00±0.22</td>
<td>84.59±0.15</td>
<td>70.38±0.34</td>
<td>82.08±0.25</td>
<td>77.59±0.42</td>
</tr>
<tr>
<td>Total lipid (% DWB)</td>
<td>7.83±0.17</td>
<td>7.46±0.21</td>
<td>5.91±0.07</td>
<td>20.62±0.21</td>
<td>6.51±0.09</td>
<td>14.12±0.31</td>
</tr>
<tr>
<td>Ash (% DWB)</td>
<td>3.64±0.03</td>
<td>3.30±0.03</td>
<td>3.32±0.02</td>
<td>3.89±0.03</td>
<td>4.01±0.04</td>
<td>3.44±0.04</td>
</tr>
<tr>
<td>NPN (% DWB)</td>
<td>2.41±0.01</td>
<td>1.58±0.02</td>
<td>2.22±0.01</td>
<td>2.36±0.03</td>
<td>2.14±0.05</td>
<td>2.46±0.02</td>
</tr>
<tr>
<td>TVBN (mg %)</td>
<td>12.00±1.73</td>
<td>12.00±2.00</td>
<td>12.00±2.00</td>
<td>14.00±1.00</td>
<td>14.00±2.00</td>
<td>12.00±1.00</td>
</tr>
<tr>
<td>FFA (% oleic acid)</td>
<td>5.50±0.30</td>
<td>2.56±0.16</td>
<td>2.50±0.19</td>
<td>4.80±0.21</td>
<td>6.35±0.18</td>
<td>3.88±0.12</td>
</tr>
<tr>
<td>TBA No (mg/1000g)</td>
<td>0.73±0.10</td>
<td>0.51±0.09</td>
<td>0.59±0.08</td>
<td>0.56±0.10</td>
<td>0.61±0.09</td>
<td>0.49±0.15</td>
</tr>
<tr>
<td>pH</td>
<td>6.50±0.12</td>
<td>6.60±0.07</td>
<td>6.50±0.15</td>
<td>6.50±0.16</td>
<td>6.40±0.18</td>
<td>6.90±0.20</td>
</tr>
</tbody>
</table>

Note: Results are mean ± S.D. of 8 samplings.

DWB = Dry weight basis.

NPN = Non protein nitrogen, FFA = Free fatty acid.

TVBN = Total volatile base nitrogen, TBA = Thiobarbituric acid.
TABLE 3. Biochemical Composition of Smoked Fishes

<table>
<thead>
<tr>
<th>Composition</th>
<th>C. batrachus</th>
<th>W. attu</th>
<th>A. testudineus</th>
<th>C. punctatus</th>
<th>T. putitora</th>
<th>P. Jayarami</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>47.58±1.50</td>
<td>5.80±0.08</td>
<td>3.65±0.03</td>
<td>5.71±0.01</td>
<td>6.00±0.01</td>
<td>15.00±0.05</td>
</tr>
<tr>
<td>Crude protein (%) DWB</td>
<td>71.44±0.35</td>
<td>79.73±0.72</td>
<td>73.74±0.40</td>
<td>76.37±0.10</td>
<td>76.04±0.54</td>
<td>83.40±0.08</td>
</tr>
<tr>
<td>Total lipid (%) DWB</td>
<td>10.49±0.10</td>
<td>7.39±0.05</td>
<td>10.25±1.26</td>
<td>3.60±0.48</td>
<td>10.73±0.42</td>
<td>4.34±0.57</td>
</tr>
<tr>
<td>Ash (%DWB)</td>
<td>4.00±0.01</td>
<td>4.50±0.01</td>
<td>7.05±0.05</td>
<td>9.41±0.05</td>
<td>5.16±0.02</td>
<td>4.43±0.04</td>
</tr>
<tr>
<td>NPN (%DWB)</td>
<td>0.83±0.01</td>
<td>3.04±0.02</td>
<td>2.51±0.07</td>
<td>1.92±0.01</td>
<td>4.85±0.03</td>
<td>3.90±0.02</td>
</tr>
<tr>
<td>TVBN (mg %)</td>
<td>40.00±0.81</td>
<td>39.00±2.00</td>
<td>24.00±1.00</td>
<td>44.00±2.00</td>
<td>40.00±1.00</td>
<td>42.00±1.00</td>
</tr>
<tr>
<td>FFA (% oleic acid)</td>
<td>5.00±0.04</td>
<td>8.65±0.03</td>
<td>4.82±0.04</td>
<td>7.91±0.01</td>
<td>7.27±0.05</td>
<td>4.40±0.06</td>
</tr>
<tr>
<td>pH</td>
<td>6.42±0.90</td>
<td>6.30±0.02</td>
<td>6.40±0.12</td>
<td>6.30±0.13</td>
<td>6.40±0.15</td>
<td>6.30±0.08</td>
</tr>
</tbody>
</table>

Note: Results are mean ± S.D. of 8 samplings.

DWB = Dry weight basis.

NPN = Non protein nitrogen, TVBN = Total volatile base nitrogen, FFA = Free fatty acid
<table>
<thead>
<tr>
<th>Fishes</th>
<th>Eyes</th>
<th>Gills</th>
<th>Texture</th>
<th>Odour</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. batrachus</em></td>
<td>Bright convex</td>
<td>Bright red to red</td>
<td>Firm</td>
<td>Fresh fish odour</td>
<td>9-10</td>
</tr>
<tr>
<td><em>W. attu</em></td>
<td>Flat but clear</td>
<td>Red to pale red</td>
<td>Firm to slightly firm</td>
<td>Fresh fish odour</td>
<td>9-10</td>
</tr>
<tr>
<td><em>L. rohita</em></td>
<td>Flat but clear</td>
<td>Red</td>
<td>Firm</td>
<td>Fresh fish odour</td>
<td>9-10</td>
</tr>
<tr>
<td><em>L. guntea</em></td>
<td>Convex</td>
<td>Bright red</td>
<td>Firm</td>
<td>Fresh fish odour</td>
<td>9-10</td>
</tr>
<tr>
<td><em>M. albus</em></td>
<td>Bright convex</td>
<td>Bright red</td>
<td>Firm</td>
<td>Fresh fish odour</td>
<td>9-10</td>
</tr>
<tr>
<td><em>A. testudineus</em></td>
<td>Bright convex</td>
<td>Red</td>
<td>Firm</td>
<td>Fresh fish odour</td>
<td>9-10</td>
</tr>
<tr>
<td>Fish</td>
<td>Eyes</td>
<td>Gills</td>
<td>Texture</td>
<td>Odour</td>
<td>Score</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------</td>
<td>----------------------------</td>
<td>--------------------------</td>
<td>----------------------</td>
<td>-------</td>
</tr>
<tr>
<td><em>W. attu</em></td>
<td>Slightly sunken &amp; cloudy</td>
<td>Pale red with slightly slime to bleaching</td>
<td>Soft leaving thumb impression</td>
<td>Neutral to slightly rancid</td>
<td>3 - 5</td>
</tr>
<tr>
<td><em>L. rohita</em></td>
<td>Flat &amp; slightly cloudy</td>
<td>Red to brown with slime</td>
<td>Slightly firm leaving no thumb impression to slightly soft</td>
<td>Neutral odour</td>
<td>3 - 5</td>
</tr>
<tr>
<td><em>L. gonius</em></td>
<td>Flat &amp; cloudy</td>
<td>Brown with slime</td>
<td>Slightly firm leaving no thumb impression to slightly soft</td>
<td>Neutral odour</td>
<td>3 - 5</td>
</tr>
<tr>
<td><em>N. chitala</em></td>
<td>Slightly sunken &amp; cloudy</td>
<td>Discolouration with mucus</td>
<td>Soft leaving thumb impression</td>
<td>No odour</td>
<td>3 - 5</td>
</tr>
<tr>
<td><em>H. ilisha</em></td>
<td>Sunken &amp; cloudy</td>
<td>Brown with slime</td>
<td>Slightly soft</td>
<td>Neutral odour to stale &amp; rancid</td>
<td>3 - 5</td>
</tr>
<tr>
<td><em>A. cor</em></td>
<td>Flat &amp; slightly cloudy</td>
<td>Red to pale red</td>
<td>Slightly firm leaving no thumb impression</td>
<td>Nutral odour</td>
<td>6 - 8</td>
</tr>
</tbody>
</table>
### TABLE 6. Reconstitution Properties and Sensory Evaluation of Smoked Fishes

<table>
<thead>
<tr>
<th>Name of the fishes</th>
<th>Reconstitution properties(%)</th>
<th>Organoleptic Properties</th>
<th>Texture</th>
<th>Colour</th>
<th>Smoky odour</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. brachycephalus</td>
<td>194.00±0.15</td>
<td>Soft</td>
<td>Black</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>W. attu</td>
<td>97.67±0.05</td>
<td>Very hard</td>
<td>Yellowish black</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>A. testudineus</td>
<td>117.67±0.05</td>
<td>Very hard</td>
<td>Black</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>C. punctatus</td>
<td>123±0.02</td>
<td>Very hard</td>
<td>Black</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>T. putitora</td>
<td>90.00±0.02</td>
<td>Very hard</td>
<td>Yellowish black</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>P. jayarami</td>
<td>142.00±0.08</td>
<td>Slightly soft</td>
<td>Light yellowish</td>
<td>+++</td>
<td></td>
</tr>
</tbody>
</table>

Notes: +++ = Pleasant, ++ = Medium, + = Poor.
DISCUSSION

Biochemical compositions of different species have been found different. The composition of fresh, iced and smoked fishes are in table 1(Fig.2.1), 2 (Fig.2.2) and 3(Fig.2.3) respectively.

Moisture

Moisture contents of different six iced fish muscle varied from 78.00 to 80.47 percent. The values were within the range as described by Love (1970). Moisture content of iced fishes are generally slightly more than that of fresh fishes. It is probably due to uptake of water during the process of icing. Garg and Stephan (1982), Perigreen et al., (1987) and Joseph et al., (1988) also reported on the increase of the value of moisture during iced storage of Kati (Pellonia sp.), Channa striatus and Labeo rohita. Moisture content of smoked fish was found to be highest in C. batrachus smoked in traditional Manipur style, while the values were vary low in other fishes (table 3).

Smoking is a process which involves preservation of the sample. Thus, water content in the finished product is quite important to determine its shelf life. In addition to this, water activity (a_w) also plays an important role. a_w is defined as the ratio of the water vapour pressure in equilibrium with the sample to the vapour pressure of pure water at the same temperature. Intermediate moisture food, with a value of a_w between 0.7 and 0.9 (water content range between 20% and 50%) does not support active bacterial growth (Karel, 1973). These foods are however, susceptible to mould growth and enzymatic dehydration unless proper prevention measures are taken. Thus, attempt should be made to lower the moisture
content of smoked fishes below 20%.

As the smoked fishes brought from outside Manipur had the moisture value between 3.65-15.00% these may be considered quite safe. Since smoked *C. batrachus* of Manipur had higher moisture content, attempt should be made to improve the product by exposing the sample longer to smoke. However, care should be taken to preserve the nutritional status of the product.

**Crude Protein (Total Protein)**

In fresh fishes protein contents varied from 70.00 to 81.28 per cent. Protein value of *L. guineea* was similar to the value reported by Sarojnalini and Vishwanath (1988a); but the value of *A. testudineus* and *C. batrachus* were less than their reported values. Protein content of *W. altu* and *M. albus* reported by Rao (1985) and Vishwanath and Sarojnalini (1988) were slightly similar with the present finding. Difference in protein content of the present investigation and earlier reports may be the factors of the difference in size of fish or the season during which the estimations were made.

Slightly lower values of proteins were seen in iced fishes. Protein content of iced *W. altu* and *L. rohita* when compared with those of fresh fishes in the present investigation (*W. altu* 81.28%, *L. rohita* 80.58%) and those reported by other workers (*W. altu*, 80.00% by Rao, 1985; *L. rohita*, 89.01% by Joseph et al., 1988) had slightly lower values. Protein contents of iced *I. gonius*, *N. chitala* and *A. aor* were also lower than those of fresh fishes reported by Jafari and Khawaja (1964). In iced fishes some protein may be lost due to leaching out of water soluble fractions from the muscle along with the ice melt water. Joseph *et al.* (1980); Garg and Stephan (1982) and Reddy and Srikar (1991) also observed decreased
value of total nitrogen during ice storage of *Chanos chanos*, Kati and Pink perch etc.

Protein contents of smoked *C. batrachus* and *A. testudineus* were less by 10 per cent and 2.5 per cent from the respective values of the fresh fishes. Similarly the content of smoked *T. pituitata* was also found to be lower than the value of fresh fish reported by Jafri and Khawaja (1964). Bhuiyan *et al.* (1986b) also observed that 10 per cent protein was lost during smoking of Atlantic mackerel. Smoking process causes loss of protein along with weight loss (Shewan, 1949a). Thus, the lower value of protein in smoked fishes may be related to - i) the technique of indigenous smoking which may not preserve the protein quality, ii) the fish which is generally used for smoking was not fresh as it should be.

**Total Lipid**

Lipid content of fresh *L. rohita* and *A. testudineus* seem to have some relationship with their moisture content, i.e. if the moisture content is low lipid content is high. Lipid content of the fishes varied from 5.74 to 12.26.

In the ice fishes lipid content has no relationship with the other components. Among the six iced fishes *A. aur* had the highest value of lipid, the next being *H. ilisha*. Some changes may occur in lipid content during iced storage. According to Fazal and Srikar (1989) phospholipid was markedly decreased with the increase of FFA content (FFA of present analysis is high). Lipid content of iced *N. chitala* (6.51%) has less value than that of the fresh one (7.086%) reported by Jafri & Khawaja (1964). Similarly the value in iced *W. attu* and *L. rohita* also has less value than fresh value given in the present investigation. Such variation in composition might be also attributed to change in season, sexual maturity and food availability (Jafri & Khawaja, *Op cit.*; Jafri, 1968).
Lipid content of smoked *C. batrachus, W. attu, A. testudineus* were low when compared with those of fresh samples of the present investigation. Depending on the smoking time, drying also cause reduction of highly unsaturated fatty acid (Taniguchi, 1988). Drying of various technological process results in substantial loss of volatile fraction of lipids, such as, short chained fatty acids and methyl esters (Colowick & Kalpan, 1969). The above reasons may be the factors for lower value of lipid in smoked fishes.

Fish lipid resembles vegetable oils and animal fats in having triglyceryl esters of fatty acids, but differs in having a variety of fatty acids of chain length from C\textsubscript{14} to C\textsubscript{24}. The principal fatty acids of animals and vegetable fats and oils contain two to three double bonds per molecule whereas fish oil contain fatty acid with four to five double bond (Jasmine, 1985).

Fish lipids are rich in polyunsaturated fatty acids particularly C\textsubscript{20:5}, C\textsubscript{22:5} (E\textsubscript{3}). There are ideal for combating hypercholestrolaemia, antheroclerosis, multiple sclerosis or coronary heart disease.

**Ash**

In the fresh state, 100 g of fish of the small species seems capable of totally satisfying the calcium need of the adult (Causeret, 1962), ice storage and smoking process cause loss of water soluble mineral elements (Devadasan *et al.*, 1978; Mc Cance & Shipp, 1933). In fresh fishes, the small fish *L. guntea* had the highest value. In all the fishes ash content were moderately high.

In iced fishes ash content was low. It was within the range of 3.30 to 4.0 per cent. Ash content of fresh *W. attu* (5.33, 9.13, 5.98) *L. rohita* (4.72, 5.02), *L. gonius* (8.18), *N. chitala*
(5.284), *H. ilisha* (5.5) given by Jafri & Khawaja (1964), Rao (1985) and present investigation fresh values have higher than the iced fishes. It may be due to - i) loss of mineral as drip during ice preservation, ii) related to the different environments in which the fishes inhabit.

Ash content of the smoked fishes varied from 4.00 to 9.4 per cent. Smoked *C. batrachus* have the same value with fresh but *W. attu* and *A. testudineus* have lesser value than those of fresh one. In smoked fishes some minerals may be lost as drip during the process. According to McCance and Shipp (1933) loss of mineral elements during processing is generally physical since liquid is discarded from the product.

**Non protein nitrogen (NPN)**

In all the iced fishes NPN values were below 2.5 (per cent on DWB). While the values were low in some fishes, it was nil in some. Iced *W. attu* and *L. rohita* have lesser values of NPN than those of fresh fishes. Garg and Stephan (1982), Basu and Khasim (1985), Perigreen *et al.* (1987) and Raddy and Srikar (1991) also observed decreasing value of NPN during iced storage of Kati, *Chanos chanos*, common murrel and pink perch, it may be attributed leaching during the process or utilization of free amino acids by the bacterial flora. NPN has significant relationship with the score of texture (Raddy & Srikar, *op cit.*).

Soluble protein nitrogen and non protein nitrogen may be leached from the muscle tissue during smoking process. In the present study, the wide range of NPN was observed. In *C. batrachus* the value was as low as 0.83 and in *T. putitora* it was as high as 4.85 per cent.
Total Volatile Base Nitrogen (TVBN)

Among the fresh fishes *L. guntea* and *A. testudineus* have TVBN value below 10.0 mg per cent. These may be considered to be quite fresh taking into account of the statement of Morris (1959). The other fishes i.e. *M. albus*, *L. rohita*, *C. batrachus* and *W. attu* were also considered to be quite in fresh condition as their values do not exceed 11.00 mg per cent.

In iced fishes TVBN value was above 10.00 mg per cent, but it was within the range of 12.00-14.00 mg per cent. According to Morris (1959), fishes with TVBN value of 10 mg per cent or less are considered quite fresh, and those below 20 mg per cent were considered satisfactory. Iced stored *L. rohita* with a TVBN value of 18.9 mg per cent (Ali et al., 1992) and ice stored murrel after 13 days with a TVBN value of 16.2 mg per cent (Perigreen et al., 1987) were declared unacceptable. The higher value of TVBN may be related to leaching of volatile bases formed during iced storage (Perigreen et al., op cit.). The values in the present are thus, considered to be within acceptable limits. According to Bandhyopadhyay et al. (1985) and Perigreen et al., (op cit.) TVBN values in freshwater fish cannot be taken as an index of spoilage on account of its low values developed during iced storage compared to tropical marine fish.

In smoked fishes TVBN values were very high. They ranged from 24.00 to 44.00 mg per cent. Lu et al (1988) did not consider TBVN value of 10.5 - 10.6 mg N per cent (DWB) to be high in smoked herrings. Joseph et al. (1987) observed the value of 38.12 and 39.86 mg per cent in 0 and 5 days smoked barracuda. According to them TVBN is not reflected in the organoleptic qualities of dried/smoked samples.
Free Fatty Acid (FFA)

The present study shows that there might be some degree of lipid hydrolysis in *W. attu* (5.50), *N. chitala* (6.35), *A. aor* (4.80) and *H. ilisha* (3.88), since the values were slightly high. FFA released during storage cause protein denaturation (Fazal & Srikar, 1989). Perigreen *et al.* (1987) observed FFA value of 6.7 on 6 days of ice stored common murrel which was 3.4 on 0 day. *Nair et al.* (1983) also observed the value of 8.00 on frozen lantern fish which was usually lower than 2.0 in the lipid of fresh fish. *Shetty et al.* (1991) also reported on increase of FFA during iced storage. This increase in FFA value during iced storage may be related to lipid hydrolysis. However, no rancid odour was observed/noted in the iced fishes of present analysis.

According to *Buiyan et al.* (1986c), the overall fatty acid composition is virtually unchanged after the smoking process. But *Allam et al.* (1988) reported that smoking lead too further increase in free fatty acid.

Thiobarbutaric Acid (TBA) Number

TBA no. of iced fishes was very low, which were below 1.0 mg/1000 gm. They were within the acceptable limit. According to *Sinhahe and Yu* (1958), TBA no. less than 3.0 mg/1000 gm sample of frozen fish is considered to be in good condition.

pH

In iced and fresh fishes pH were slightly acidic. In smoked fishes pH were more acidic, which might be due to the phenolic/acidic constituents deposited on the fish muscle during smoking. The delay in the onset of fungal attack in smoked fish can be attributed to the anti-fungal action of the smoke constituents deposited on fish (*Joseph et al.*, 1987).
Sensory Test

Sensory parameters give a reliable result on the self life of the fishes. The shelf life of Fanghn's Mackerel on ice was reduced by one day for each hour of delay in icing or exposure to ambient temperature of 28°C -30°C (Barile et al., 1958). Bandhyopadhyay et al. (1985) reported that bigger sized fish had better ice storage life than smaller ones e.g. shelf life 30-33 cm long fishes had 17 days.

Carps had a slightly longer storage life than catfish in ice (Devadasan et al., 1978). Under similar condition of icing, fish out of contact with ice melt water have longer shelf life and more acceptability than the fish preserved in contact with ice (Basu & Khasim, 1985). In iced condition the acceptability of murrel was 8-9 days (Perigreen et al., 1987), of rohu : 12-15 days (Joseph et al., 1988; Ali et al., 1992) of Chanos chanos : 12-14 days (Joseph et al., 1980) of Kati : 9 days (Garg and Stephan, 1982).

In all iced fishes organoleptic quality was not so good. The technological problems of handling and transportation of Indian freshwater fish are less formidable as compared to those associated with sea fishes. According to Govindan (1985), it takes 1-2 days for the ice fishes to reach Calcutta. By the time it reaches the city, a high percentage of the fish is of sub standard quality due to insufficient icing. It takes another 4-5 days to reach different marketing centre of north eastern states where no proper icing facilities are available. Thus, the iced fishes sold in Manipur are of not of very high quality.

Organoleptic conditions of the fresh fishes are in very good stage. In smoked fishes smoked odour were medium to pleasant. Except P. jayarami and traditionally smoked Clarias, all were of very hard texture.
Storage/ Processing Quality

Use of wood smoked with its antioxidant properties is more ideal than synthetic chemicals for controlling rancidity, particularly its phenolic content, which must have also contributed to the good taste, flavour and colour (Chandrasekar et al., 1979). Smoked products are stable only if sufficient to lower the water activity to a level which would not support microbial activity (Chan et al., 1975). Fishes smoked in conjunction with salt, sorbate, dehydration and low storage temperature has been found more effective in reducing spoilage than use of liquid smoked alone (Horner, 1992). Hot smoking murrel at 70±5°C for 5 hours had self life of 6 days at room temperature (Hanumanthappa & Chandrasekher, 1987). Kasahara and Nishibori (1981a,b) found that carbonyls, alcohol and phenol were the chief volatile components of smoking fish. Thus, smoking has preservative effect.