CHAPTER-IV

Estimation of proximate composition
CHAPTER 4

ESTIMATION OF PROXIMATE COMPOSITION

INTRODUCTION

Today one among the most important health problems of the world is malnutrition. It is due to lack of ability to buy enough food, production, and use of foods of low nutritive value. Fish is a best alternative to this. Fish has special importance as a supplementary food item to ill-balanced cereal diets. Analysis of the food consumption tables reveal that the average diets are generally satisfying from the view point of their energy content. Food intake are generally defective in their nutritional quality since they are rich in carbohydrate which may contribute 70-90% of total caloric intake and too little protective food, rich in protein and other nutrient leading to nutritional deficiency. The main deficiency affects the large number of people is protein caloric malnutrition. This can be overcome by increased consumption of fish as a well balanced protein diet.

The nutritive value of fish has been recognised from time immemorial. While considering fish as a good source of food because of its body with a dominance of muscular
tissue the main emphasis is put in its protein content. The Principal constituents of fish are water, Protein, Fat and Carbohydrates. Fish proteins are having its own importance because it contains all the essential amino acids and are said to be easiest to digest. The protein digested and assimilated by the fish are mostly incorporated into muscles of the fish. The studies on the growth, body weight, maintenance, involving nitrogen balance techniques and the rate of regeneration have confirmed the fact that fish contains proteins of a high biological value with maximum digestability co-efficient. Fats, on the other hand have a high caloric value and are stored mainly in muscles and liver.

The fresh fish flesh is there fore a good source of food, which provides an excellent nutritive value for human diet. Various studies have been carried out on the proximate composition of the flesh of fishes and a perusal of the previous literature reveal that variability in the bio-chemical composition of the fish tissues are very common. This is mainly due to many factors such as food, season, developmental stages and environmental conditions. Siodigi (1966) reported that in Ophiocephalus punctatus the total cholestral content shows variations from season to season. Jafri (1968 a,
1968 b and 1969) worked on different fishes and stated that marked changes occurred in the bio-chemical composition of the fishes from season to season. Kalpana D.Shreni (1980) confirmed the above views by working on Heteropneustes fossilis. On the observations of Masurekar and Pai (1979), Divakaruni and Sharma (1986) it is clear that the bio-chemical composition of the fish shows some variations during their developmental stages.

Gupta and Joshi (1982) studied the effect of temperature on the carbohydrate constituents in Clarius batrachus.

The Proximate composition study will give the clear picture of the nutritive value of the fishes and their suitability and importance of consumption. It is a known fact that the bio-chemical composition of the fishes grown in polluted water shows variations. Even though every living organism is having its own detoxification mechanisms to get rid off from foreign substances, the heavy effect of pollutants bound to bring severe adverse effects to the animal. Because of this the fishes grown in polluted waters shows variations in their nutritive value and some times the heavy pollutants made them toxic also. Studies carried out on fishes grown in polluted water showed changes in total protein, hepatic and blood cholesterol. (Dubale and Mohini Awasthi 1982,
Kamble 1983, Bhattacharya et al 1984). The data available on the chemical composition in relation to nutritive value of fresh water fishes grown in industrial effluents is meagre. Hence the present study is carried out which is an attempt to find out whether the body chief constituents such as protein, carbohydrate, fat and water of the muscles of the economically important fresh water fish Labeo rohita shows any changes when grown in different safe concentrations of distillery effluents.

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MATERIAL AND METHODS

As the objective of the study is to find out the changes if any in the chemical composition of the muscles of the fish *Labeo rohita* grown in different concentrations of distillery effluent, the proximate composition study was made in the fish flesh before starting the experiment and after completion of the growth study in the effluent and also in the control.

The fishes brought for the experimental study were acclimatized and before starting the growth study in the various concentrations of distillery effluent, few live fish were stunned with a blow and bled. The scales, skin, and bones were removed and only the flesh was used for the analysis of the nutritive value. Similarly after completion of the growth study experiment in different distillery effluent concentrations, from each concentration few fishes were killed and only flesh was used to find out the bio-chemical composition. Simultaneously the proximate composition was analysed in the fish flesh grown in the control also. The chemical composition analysis were done for total protein, Carbohydrate total fat and water content.
Estimation of Protein: (Gornel et al 1949)

The dried muscle tissue was analysed for protein by the biuret reaction method. The biuret solution was prepared by dissolving 3 g of CuSO₄ and 9 g of sodium potassium tartarate in 500 ml. of 0.2 N Sodium hydroxide solution. To this solution 5 g of potassium iodide was added and the volume was made up to 1000ml with 0.2 N Sodium hydroxide solution. Bouine serum albumin was used as standard.

The muscle strips free from scales, skin and bones of the entire fish was taken and dried in a oven. 10 mg of the powdered dried tissue was taken in a test tube to which 2 ml of distilled water was added, followed by 6 ml of biuret reagent. At room temperature the solution was mixed well. After thirty minutes the optical density was measured in ErnaColorimeter with 520H filter using the reagent blank. The protein was estimated by comparing with standard graph.

Carbohydrate estimation: (Dubios et al 1956).

10 mg of the scale less, skin and bones free muscle powder was taken in a test tube to which 1 ml of distilled water was added followed by one ml of 5% phenol and 5 ml
of analar sulphuric acid (Sp.gr. 1.835). After some
time 4 ml of distilled water was added. The solution
was allowed to stand for 30 minutes and then mixed
throughly. After cooling the solution the intensity of
the colour was read in the calorimeter using 490A filter
using the reagent blank. Carbohydrate in the sample
was estimated by using standard graph. The standard
solution was prepared by using sucrose.

Estimation of Fat: (Bligh and Dyer Method)

The fat was extracted using 2:1 mixture of
Chloroform and methanol. One g wet weight of the skin
and bone free tissue was grounded in a pestle and
mortar with 10 ml of dis. water. The pulp was transfered
in to a conical flask of 250 ml capacity and to which
30 ml of chloroform-methanol mixture was added. After
thorough shaking for complete extraction it was kept
open over night at room temperature in the dark. Next
day a further 20 ml chloroform was added. The result-
ting solution showed a clear lower layer of chloroform
containing fat. The upper layer was discarded and the
lower layer was transfered into a weighed container for

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Estimation of Water:

The water content of the body of the fish was estimated by drying in even and measuring the weight loss.

The whole fish weight was taken by keeping in a weighed watch glass after removing the adhered water on the body. The fish was kept with the watch glass in a hot air even thermostatically controlled at 101°C for 24 hours. Then allowed to cool at room temperature and the weight was taken.

The water content percentage was calculated as per Aitkan et al (1979)

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\text{Water Content} = \frac{\text{Weight of Wet fish} - \text{Weight of dried fish}}{\text{Weight of wet fish}} \times 100
\]

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RESULTS AND DISCUSSION

Fish remain today as one of the cheapest source of food and animal protein so far as man is concerned. The share of fish as a percentage of animal protein intake by poor people is much larger in developing countries than in developed countries. Therefore fishes have a special relevance so far as the developing countries are concerned as they are excellent sources of easily digestable proteins and certain essential minerals, vitamins and fats.

A critical assessment of the bio-chemical composition variability of tissues of the fish is essential in order to understand the effect of the distillery effluent concentrations on the flesh of the fish. Hence the present work has been carried out on the proximate composition of the flesh of the fish *Labeo rohita* grown in different concentrations of distillery effluent. The result has been compared with that of the control fish and to that of the pre-experimental fish. The results have not shown any adverse effect on the proximate composition of the flesh of the fish after exposure to different effluent concentrations and have been given in table No.71.
The estimation of protein in the flesh in control fish after growth study showed a decrease in protein level when compared with pre-experimental fish. It is 21.5% in pre-experimental fish and 19.1% in the control fish after the experiment. This may be due to the growth of the fishes under captivity or due to the non-availability of other natural foods, as only artificially prepared pelleted feed has been fed during the experiment. The protein level increased from 19.1% to 21.5% in 1% concentrations respectively. This throws light on the fact that the distillery effluent concentrations have no notable adverse effects on the protein content of the fish flesh. The results shows that there is slight variations in the protein value in some concentrations and it may be due to more food intake in that concentration or the hydrological parameters. This can be evidenced with the result that in 0.5% concentration the protein level is 21% in which the food consumption and conversion efficiency were maximum when compared with other concentrations. Even though there were slight variations in the protein value, the variations were negligible and it is also noticed in control fish as well as in pre-experimental fish. It clearly shows that
the concentrations of the distillery effluent have not affected the protein content of the fish flesh. (Fig.6).

The outcome of the analysis of total carbohydrate in the experimental fish flesh showed that the carbohydrate level is almost similar in the flesh of fish grown in different effluent concentrations and control. In the pre-experimental fish it was 11.5% which almost tallies with the value of fishes grown in different concentrations, except in 0.5% with 12% carbohydrate. As there is no change or decrease in carbohydrate level in the fish flesh grown in the effluent and control it confines the fact that the distillery effluent concentrations have not played any role in the carbohydrate content of the fish. (Fig.7)

The values obtained in the estimation of total fat in the fish flesh after growing in different effluent concentrations and also in control showed very less percentage. The fat content is seen to decreased from 1.2% in the pre-experimental fish flesh to 0.82% in the control fish and to an average of 0.84% in the flesh of the fish grown in all other different distillery effluent concentrations. The value is seen to the almost uniform in all the concentrations and control fish. (Fig.8).
The result evidences the fact that the effluent concentrations have not influenced the fat value in the experimental fishes as the value decreased in the control fish also. It may be due to the non availability of natural food material, as the fishes were fed only with artificially prepared pelleted food. It is observed that the fat content is more in pre-experimental fishes than in the fishes grown for a period of twelve months.

The moisture content estimation was made in the fish before the experiment, in the fishes grown in different distillery effluents and also in the control, fish. The moisture content was 76.6% in the pre-experimental fish and it decreased to 75.1% in the control fish. The water content was almost uniform (average 73.4%) in all the fishes grown in different distillery effluents but shows a tendency to decrease slowly from lower concentration to higher concentration (Fig. 9). The reason for the decrease of moisture content in the fishes grown in distillery effluent in relation to higher concentrations and also in control fish are not known.

Damborg (1964) noted that the decrease of protein (-5%) in the cod muscles at the time of spawning, he also given the correlation between the seasonal variation and
the physiological process taking place in the animals body. Masurekar and Pai (1979) observed that in *Cyprinus carpio* the protein content decreased gradually with the advance of gonad maturation. According to Akolkar and Salsara (1984) the variation in the protein in the flesh of *Cyprinus carpio* seems to be influenced by growth rates and environmental factors and his results showed an increase of protein value in relation to age. This result is not in tally with the above work as the maturity is mainly in relation to age and growth. In the present study on the protein content there is no much variations and the slight variations may be due to the influence of environmental factors. Kamble and Keshavan (1986) observed that there are changes in the muscle protein content of *Barilius bendelisis* when treated in lethal concentrations of sevin at 96 hour. But in the present work no such variations were observed and it shows the non-lethal effect of the distillery effluent on the protein content.

Gupta & Joshi (1982) observed that the carbohydrate contents of *Clarius batrachus* changes due to the temperature changes. In the present study as the experimental temperature was kept constant there is no influence on the carbohydrate level. The increase may be due to the
growth of fish, which corroborates with the work of Akelkar and Balsara (1984) in *Cyprinus carpio*.

Rao (1967) worked on the fat content of the *Pseudorasora alpina* and found that the fat content decreases at the time of spawning. Damberg (1964) confirmed it by working on cod fillets that the fat contents are highest in immature fishes. The fat content values observed in this work for the *Labeo rohita* grown in different distillery effluent concentrations is in tally with the above work.

According to Damberg (1964) the water content in the muscles of cod fish increases with the progress of maturation. It was confirmed by the work of Masurekar and Pai (1979) by working in *Cyprinus carpio*. As per the result of Akelkar and Balsara (1984) an increase of moisture content up to certain level was observed in *Cyprinus carpio* and then decreased. No explanation have been given for the decrease in the water content. The results of present work gave on entirely different picture, but the decrease in moisture content level after growing for a period of twelve months is in tally with the latter's work. The uniformity of moisture contents in the fishes grown in different distillery
effluent concentrations and the slow decrease with the increase of concentration may be due to the influence of the environment in which fish has grown.

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<table>
<thead>
<tr>
<th>Concentration</th>
<th>Protein (%)</th>
<th>Carbohydrate (%)</th>
<th>Fat (%)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-experimental fish</td>
<td>21.5</td>
<td>11.5</td>
<td>1.2</td>
<td>76.6</td>
</tr>
<tr>
<td>Control</td>
<td>19.1</td>
<td>11.0</td>
<td>0.82</td>
<td>75.1</td>
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<td>0.1%</td>
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<td>11.0</td>
<td>0.84</td>
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<tr>
<td>0.25%</td>
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<td>73.5</td>
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<td>0.5%</td>
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<td>12.0</td>
<td>0.86</td>
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<tr>
<td>1%</td>
<td>21.5</td>
<td>11.5</td>
<td>0.83</td>
<td>73.1</td>
</tr>
</tbody>
</table>
Fig. 6

Protein value in the flesh of *Labeo rohita* grown in different distillery effluent concentrations and control.
Protein Value

X Axis 1CM: 0.1%

Y Axis 1CM: 0.10D

Fig: 6
Fig. 7
Carbohydrate value in the flesh of *Labeo rohita* grown in different distillery effluent concentrations and control.
Carbo-hydrate Value

X Axis 1CM: 0.1%
Y Axis 1CM: 0.2 OD

OD Value

Cone. of Dist. Effl.
Fig. 8

Fat value in the flesh of *Labeo rohita* grown in different distillery effluent concentrations and control.
Fig. 9

Water content of *Labeo rohita* grown in different distillery effluent concentrations and control.
Water Content

X Axis 1 CM: 0.1 %

Y Axis 1 CM: 10 %

Fig: 9