CHAPTER - I

3. PROXIMATE COMPOSITION OF *M. CORDYLA* AND *O. RUBER*

3.1 Introduction

Fish is a major source of animal protein and it also contains several important minerals and vitamins essential for human beings. Fish meal is widely consumed in many parts of the world because of its high protein content, low saturated fat *etc*., which support to maintain good health. And more over fish lipids are rich in long chain n-3 polyunsaturated fatty acids (PUFA) and play an imperative role in human nutrition, disease prevention and health promotion [93, 94 & 95]. The marine fishes are rich source of nutrients, however, the chemical composition and nutritional levels of fish varies greatly from one species [96, 97] and one individual to another depending on age, sex, environment and season [98, 99 & 100]. Actually, the variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning. Processors have a direct interest in the chemical composition of fish, needing to know the nature of the raw material before the techniques of chilling, freezing, smoking or canning can be correctly applied [101]. Furthermore, chemical composition, freshness, appearance, odour, flavour and

microbiological condition are the more important factors that determine quality of fish [102]. So, complete study on fish is needed especially the information of organ wise proximate composition *viz*., evaluation of lipid, protein and fatty acid composition.

India is one of the largest marine fish producers in the world. The yield of marine capture production in 2005 was 84.2 million tons, accounting 59.5% of global production. Commonly available marine fishes in India includes ribbon fish, croaker fish, eel fish, horse mackerel, katti fish, leather jacket, mahi mahi. With the rapid development of fishery, various processing companies were established in and around the coastal areas. Moreover during harvest season, many fishes are discarded, due to not-processed on time. Hence, comprehensive utilization of marine fish, especially the production of value-added products has both environmental and economical importance. Most of these marine fishes are commercially important and highly consumed without knowing their medicinal importance. Since the Tamilnadu coastal line has good fishery resources, the *Magalaspis cordyla* (horse mackerel) and *Otolithes ruber* (croaker) were selected for present work. Studies on these fishes are very limited and especially the information of organ wise proximate and fatty acid composition is lacking. Present study investigates the proximate analysis including moisture, ash, protein and lipid content along with fatty acid composition using gas chromatography technique from four different body parts of two fishes.

3.2 Material and Methods

Bovine serum albumin (BSA), alkaline copper sulfate reagent, folin's reagent, chloroform and methanol were purchased from Himedia Pvt., Ltd., India.

3.2.1 Proximate Analysis

a. Moisture content

The moisture content of the fish tissues was determined according to the methods of AOAC [103]. Raw sample (5g) of skin, muscle, bone and visceral mass of *M. cordyla* and *O. ruber* was weighed and incubated in an incubator at 100 ºC for 28 hours. After 28 hours the samples were removed and weighed. The difference in weight gives the moisture content of the sample. The moisture content was expressed as percentage (%).

Moisture content (%) = (Initial weight – Final weight/ Initial weight) × 100

b. Ash content

The ash content of the fish tissues was calculated according to AOAC [103]. The dried samples of skin, muscle, bone and visceral mass were weighed and placed in crucibles. These crucibles were then placed in a muffle furnace at 420 ºC for 3 hours. Then the crucibles were removed and the ash obtained was weighed. The ash content was expressed as percentage (%).

Ash content (%) = (Initial weight – Final weight/ Initial weight) × 100

c. Protein content

The protein content of the skin, muscle, bone and visceral mass of *M. cordyla* and *O. ruber* was assayed by the method of Lowry *et al.,* [104]. The stock solution was prepared by dissolving 1mg of Bovine serum albumin in 1ml of distilled water. From which different concentrations of the standard (0.2- 1.0 ml) were taken in different test tubes and made up to 1ml using distilled water. 4.5ml of alkaline copper sulfate reagent was added in each test tube and the solutions were mixed well. The solutions were incubated at room temperature for 10 minutes. 0.5 ml of reagent Folin Ciocalteau solution was added to each tube and incubated for 30 minutes and then absorbance was measured at 540 nm. The absorbance was plotted against protein concentration to get a standard calibration curve. The protein content of the fish samples was determined by using this standard curve. The protein content was expressed as percentage (%).

d. Lipid content

Lipid content was estimated by Bligh and Dyer [105] method. Fifty grams of samples of skin, muscle, bone and visceral mass of *M. cordyla* and *O. ruber* were homogenized by adding 40 ml of water followed by chloroform and methanol (1:2) and vortexed well for 10 min. To this mixture again chloroform and water (1:2) was added and centrifuged at 1000 rpm. Bottom phase was recovered and evaporated at 60 °C in a water bath and isolated

lipids were collected and measured. The lipid content was expressed as percentage (%).

Lipid content (%) = (Initial weight – Final weight/ Initial weight) × 100

3.2.2 Statistical analysis

All the assays were conducted with three replicates and the data was expressed as mean ± standard deviation. The statistical analysis was performed using statistical package for the social science (SPSS) 10.0 software (SPSS Inc. Chicago, IL, USA). The significant difference was determined with 95 % confidence interval ($P<0.05$).

3.3 Results

Freshly collected fishes were purchased from the market, brought to the laboratory and washed twice with running water followed by double distilled water. The data present in the table (1) shows the proximate composition of different body parts of two fish's *M. cordyla* and *O. ruber* were studied. The moisture content of these fishes ranged in between 73.7 – 83.8% and both the species showed maximum content in muscle than remaining body parts. Ash content was also estimated from all the body parts and their percentage was in between 1.4 to 2.6 % in muscle, visceral mass and skin. But, bone showed a high content of ash with a maximum of 12.5% in *M. cordyla* and 11.4% in *O. ruber*. The protein content in the current observations was quite high in skin

(13&14.3%) and muscle (16.5 & 12.7%) compared to remaining body parts. The relative lipid content varied from one fish to the other, but all the body parts of two fishes showed lipid values below 4.8% in the present study. As per the obtained results *O. ruber* showed good quantity of lipid than that of *M. cordyla*. As tabulated (1) high lipid content was observed in the bone of *M. cordyla* (3%) and *O. ruber* (4.5%) which was found to be interesting. Since, bone being waste this can be used for production of fish oil which will have both environmental and medical significance.

Table 1 Proximate Composition of various body parts of *M. cordyla* (MC) and *O. ruber* (OR)

	Muscle		**Visceral mass**		**Skin**		**Bone**	
(% w/w)	**MC**	**OR**	**MC**	**OR**	**MC**	**OR**	**MC**	**OR**
Moisture	80.4±0.7	83.8±1.1	83.6±1.7	81.2±2.5	83.5±0.6	79.5±1.5	73.7±0.4	75.3±2.0
Ash	1.7±0.8	2.1±0.8	2.6±0.7	1.4±0.4	1.5±0.2	1.4±0.1	12.5±2.2	11.4±2.3
Protein	16.5±0.7	12.7±1.9	11.7±0.5	13.1±2.2	13.0±3.6	14.3±2.5	10.8±4.8	8.9±2.5
Lipid	1.4±0.3	1.4±0.3	2.5±0.3	4.3±2.1	2.0±0.1	4.8±1.2	3±0.1	4.5±0.5

Values are Mean ± S.D., n=6

3.4 Discussion

Proximate composition is a good indicator to understand the physiological condition and quality of a fish but it is relatively time consuming to measure [106]. It includes the analysis of moisture, ash, protein and lipid contents of fishes studied. Carbohydrates and non-protein compounds are present in negligible amount and are usually ignored for routine analysis [107]. The percentage of water is good indicator of its relative contents of energy, proteins and lipids. The lower water content, pays the way to greater lipid and protein contents, which indeed higher the energy density of the fish [108]. However, these values vary considerably within and between species, size, sexual condition, feeding season and physical activity. Protein content, which is important component, tends to vary little in healthy fish [109]. Extensive literature is available on body composition of various fish species [110-116], which helps us to understand the basic quality of a fish.

Generally, marine fishes consist of around 65 to 80% of moisture content in their body and current findings are also in the same direction (table 1). The moisture content in other fishes like *Mugil cephalus* (79.1%) and *Harpodon nehereus* (89.8%) had more moisture content than *M. cordyla* and *O. ruber* but other marine fishes like *E. volitans* (62.3%) [39], *P. niger* (74%) [44], *Scomberomorus guttatus* (70%), *Hilsa hilsa* (72%) and *Sparus aurata* (63.5%) [117] showed more are less similar results. Ash is one the constituent in the food that doesn't burn up. After burning, the residue that is left is ash.

This method of analyzing feeds is a bit anachronistic, because more sophisticated methods of analysis are available now. Most of the material that makes up ash in muscle is unabsorbable by the animal, so "ash" is considered as waste in animal feeds, and low ash content is considered good. In the muscle of *M. cordyla* and *O. ruber* the ash content was around 1.4-2.1%, which was very high when compared with the fresh water clam (0.3%), eel (0.4%) and large yellow croaker [118, 119]. But similar ash content was reported from muscle of *N. japonicas* (1.7%) and *E. volitans* (2.1%) [38].

Fishes evolved a number of strategies to allocate energy stores to maximize survival, growth, and reproductive success. The relative amounts of protein and lipid utilized for growth to avoid predation as opposed to that stored as energy reserves can have profound effects on recruitment [120]. As Chandrasekhar and Deosthale [117] recorded the protein content ranges from 8 to 21% in marine fishes and 13 to 17% in fresh water fishes, both the fishes in the present study showed within the range. Percentage of protein content of these fishes were in the line of *Macrourus berglax* (16%) and *Centroscyllium fabricii* (17%) [121]. whereas *O. ruber* visceral mass (4.3 %) and skin (4.8%) showed high lipid content which was very high than *P. niger* (3.9%) [44], *Pampus argenteus* (1%), *Notopterus notopterus* (1%), *Nibea soldado* (1.5%) [122-124] on the other hand the lipid content is same as in *Alepocephalus bairdii* (3%), *A. agassizii* (3.6%) [121]. Based on the moisture and lipid contents, the *O. ruber* is a medium-fat fish, with a lipid content of 5–10% by

weight [125]. According to Feeley *et al*., [126], low-fat fishes has higher water content, and as a result, their flesh is white in color. Fatty fish store the fat in muscle tissue, and their flesh may be yellow, grey, pink or another color [127]. Fat content is influenced by species, season, geographical regions, age and maturity [128]. The total proximate composition of the muscle, visceral mass, skin and bone are showing some remarkable variation. *M. cordyla* and *O. ruber* contains good percentage of lipid which was very high than *N. japonicas* (1.6%) [37]. The result matches, with rainbow trout [114], which has been considered to be nutritional important species since they have a relatively high content of omega-3 fatty acids. Marine fish origin proteins and lipids play a major role in the human diet. And the results from the present study showed high moisture and protein content along with the moderate lipid content in all the four different body parts. So to elevate and improvise the applications of fish protein, the study was planned to isolate antioxidant peptides and collagen which are beneficial for human health.