INVESTIGATION OF BIOCHEMICAL ASPECTS IN EDIBLE TISSUES OF FLATFISHES ALONG THE COASTS OF BALUCHISTAN AND SINDH

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ABSTRACT

The current study was based on proximate composition of seven species of sole fishes belong to three different families; Paralichthyidae, Cynoglossidae, and Soleidae. Biochemical analysis have been done on *Cynoglossus punticeps, Cynoglossus arel, Pseudorhombus javanicus, Pseudorhombus elevates, Cynoglossus quadrilineatus, Brachirus orientalis, Dagetichthys commersonnii*. Biochemical composition was analyzed and compared along five landing sites from Balochistan and Sindh province. Current study revealed that the high Protein concentration was estimated within species of from sole fishes, the highest reported value was 664.969mg/mL from *Pseudorhombus elevates* and lower was reported 25.723 mg/mL from *Dagetichthys commersonnii.*
Lipid concentrations have been done within species whereas highest lipid was 36.198% in *Cynoglossus puncticeps* and lowest found 7.613% in *Dagetichthys commersonnii.* While highest carbohydrate found 5.277% in *pseudorhombus javanicus* and lowest was 3.8722% in *p. elevatus.* Highest moisture content was reported 75.433% in *p. elevats* and lowermost was 70.839% in *Brachirus orientalis.*

Keywords: Biochemical analysis, Flatfishes, Carbohydrate, Lipid, Proteins, Moisture.

INTRODUCTION

Food is an important component of human health because the quality and quantity of food components have a direct effect on the health status of consumers (Bamji, 2011; Paul *et al.*, 2019). Seafood is one of the best sources of meat quality and nutrient accessibility and availability relative to other animal protein products and it is ever such cheap as compared to other meat (Louka *et al*. 2004; Mohanty *et al*., 2019). Fishes seem to be the good natural sources of nutritional, protein and vitamin (Still and Robinson, 1980; Dutta *et al.*, 2018). Lipids and carbohydrates are considered as the main nutrients in the fish diet, providing the energy needed to optimize growth (Gaxiola *et al*., 2005; Akbarzadeh *et al*., 2019). Due to the important role of fish and seafood which balance protein, lipid, vitamin, minerals and have a moderately low caloric value than other muscle foods, human nutrition and health is blessed. The flesh of hilsa is a standard source of proteins in human diet and value by maximum of the fish-eaters by dint of its tasteful pleasure and smell. The biochemical composition is measure of fish excellence of fish physiological state (Ali *et al*., 2005). A decent dietary amount of lipids and carbohydrates levels and sources affect the efficiency of their use (Lovell *et al*., 1998: Akbazadeh *et al*., 2019). Fish meat provides energy and high quality of proteins, which holds all mandatory amino acid in effortlessly digestible thus, they are worthwhile nutritious sources (Weatherley and Gill, 1998) and consumed extensively worldwide. It provides polyunsaturated fatty acids specifically ω-3 fatty acids likewise docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are excessive concentrated to brain which are required for visual sharpness, cognitive function and early development (Birch *et al*. 2000). Marine fish and shellfish are ever so valuable products of seafood and have risen approximately 8%of annual human consumption economic growth (Glamuzina *et al.*, 2009; Bogdanovic *et al*., 2014; Saher and Kanwal, 2019). Marine fishes are cheaper and abundant as compared to fresh water fishes (Silva *et al*., 2009). Owing to the rising cost of animal prdocuts, consumers have become more and more interested in fish as a source of dietary protein (Emere and dibal, 2013). Fish as a whole has a great deal of food resources and can therefore expected to alleviate malnutrition , particularly in developing countries (Ashraf *et al*., 2011) it accounts for around 60% of total protein intake in adults, especially in rural areas (Adeleye, 1993; Ayeloja *et al*., 2020). Proximate composition of *Solea senegalens* was investigated in Spain and the mean value of carbohydrate was observed with 44.3% whereas protein value with 21.7% (Rodiles *et al*., 2012). Biochemical composition was investigated from the muscles of Sole fishes and moisture content was reported from *C. nigrodigitatus* with 66.19%, 5.3% Ash, 7.67% Crude fiber, 14.2% Crude protein, 10.62% Fat, 3.69% Carbohydrates while in *C. senegalensis* moisture content was reported with 63.97%, 7.7% Ash, 10.71% Crude fiber , 18.56% Crude protein, 2.57% Fat, 7.26 % Carbohydrates whereas in P. quadrifilis moisture content was observed with 67.547%, 5.94% Ash, 9.51% Crude fiber, 17.83% Crude protein,

3.39% Fat, 5.30% Carbohydrates. All three fishes had different proximate composition as they were compared in Nigeria (Daniel, 2015).Moreover it is necessary to measure the biochemical composition such carbohydrate, moisture content and protein content and lipids percentage to know the meet value and for commercial purposes (Watermann, 2000; Fawole, 2007).

MATERIALS AND METHODS

A total of 98 specimens of flatfish were collected from five different harbors in Pakistan including Sindh and Balochistan coasts to perform biochemical composition such as Protein, lipid, Carbohydrates and Moisture content. For protein estimation Bradford (1976) method was used. Firstly Fish tissue was taken in petri dish dried in oven on 65^{o} C for 24 h Later dried and crushed by grinding machine. Afterward 0.1 g of crushed sample was taken in the test tube and 10 ml of distilled water were added and kept at room temperature for 5 hours. After 5 hours each test tube was vortex to mix it properly. Formerly 2.5 mL of Bradford reagent was added to each test tube. At the end of this process absorbance was measured at 595nm using Spectrophotometer. The Folch *et al*. (1956) procedure was used to estimate the total lipid content. 200 mL chloroform, 100 mL methanol and 0.5 g dried meat of fish were taken and 10 mL solution added for further process and kept them 24 h room temperature. W1 dried (dried flask) Filtered solution in dried flask (W1) Evaporated in oven on 100 ^{o} C until it dried. Dried flask has taken dried material (lipid) is W2. While W1-W2 = W3 so W3 is our lipid concentration which has been measured. The Dubois *et al*. (1956) procedure was used to estimate total carbohydrates in fish sample. 0.1 dried meat sample taken and 10 mL of distilled water added. Phenol and sulfuric chemicals were used to determine the total lipid concentration in muscles of fish. One mL sample solution of carbohydrate has been placed in test tube to analyze, Five mL phenol, 95 mL distilled water and 1 mL sulfuric acid was added in the solution. The solution turns orange-yellow or dark shading is as aftereffect of association between the phenol and carbohydrates. The absorbance at 490 nm was measured in carbohydrates. Moisture content of sole fishes have been measured by (Saher and Kanwal, 2019) method where wet tissue was oven dried at 65ºC kept for 24 h the difference between first weight and second weight considered as total moisture content than estimated them in percentage.

RESULTS

Proximate determination

Biochemical composition analysis is very significant and is ever such importance for nutritional consideration and evaluation (Saher and Kanwal, 2019). The chemical composition of the fish differs greatly, according on age, sex, climate and season, from one species to the next. Knowledge of the biochemical composition of fish species is of crucial importance for the implementation of various technical processes (Stansby, 1967; Connell 1975; Huss, 1988; Yeannes, 2003). In current study proximate composition i.e. (Lipid, protein, carbohydrates and Moisture content) of seven sole fish species was analyzed.

Lipid Concentration

Maximum lipid content was found in *C. punctieceps*, whereas minimum lipid found in *D. commersonnii* within species (Table 1). Lipid content was also analyzed in according sites where maximum lipid found 65.448% in *C. punctieceps* from Ormara coast while minimum was 4.46% in *C. arel* from Pasni coast (Table 2).

Carbohydrate Concentration

The highest carbohydrates concentration found in *P. javanicus*, while the lowest was found in *C. punctieceps* within species (Table 1). Within sites highest carbohydrate reported 6.093% in *P. javanicus* from Ormara fish harbor whereas lowest was found 2.940% in *Brachirus orientalis* from Karachi fish harbor (Table 2).

Protein Proximate

Highest protein concentration was found in *P. elevates* and lowermost present in *D. commersonnii* within species (Table 1). Within sites highest reported value found 807.812 mg/ml in *P. javanicus* from Ormara fish harbor and lowest value was 25.688 mg/ml in *C. puncticeps* from Karachi fish harbor (Table 2).

Table 1. Descriptive statistics (Mean ± Standard deviation, Minimum and Maximum) of biochemical constituents in the muscles of various edible fish species from coastal waters of Pakistan.

Fish species	Lipid%	Carbohydrate%	Moisture%	Protein mg/ml
C. arel	18.811 ± 13.063 (2.9–51.8)	4.978 ± 1.022 (2.352–6.425)	72.754 ± 3.328 (64.919–78.758)	202.595 ± 156.246 (26.992–615.584)
C. quardilintus	12.925 ± 8.421 0.02–30.36	3.770 ± 0.576 2.487–4.937	73.110 ± 2.955 68.804–79.865	372.719 ± 353.3824 3.103–1126.127
C. punctieceps	36.198 ± 67.109 (4.06–224.4)	3.2949 ± 0.462 (2.358–3.951)	71.174 ± 2.094 (65.979–72.921)	144.008 ± 182.961 (18.761–557.632)
B. orientalis	7.984 ± 8.076 (1.8–36.42)	4.004 ± 1.285 (2.752–6.45)	70.839 ± 10.490 (55.896–99.996)	197.587 ± 219.048 (7.720–670.806)
D. commersonnii	7.613 ± 5.046 (3.06–13.04)	4.264 ± 0.806 (3.333–4.748)	74.276 ± 0.68 (73.536–74.889)	25.723 ± 10.521 (14.207–34.832)
P. javanicus	12.534 ± 9.806 (3.66–32.56)	5.277 ± 1.281 (3.215–8.25)	72.139 ± 2.855 (67.621–76.767)	389.415 ± 389.971 (10.118–1145.027)
P. elevates	13.632 ± 5.926 (6.72–22.38)	3.8722 ± 5.020 (3.198–4.521)	75.433 ± 4.909 (70.317–82.325)	664.969 ± 332.142 (356.374–1196.785)

Table 2. Descriptive statistics (Mean ± Standard deviation, Minimum and Maximum) of spatial variations in biochemical constituents in the muscles of various edible fish species from coastal waters of Pakistan.

Sites	Fish Species	Lipid (%)	Carbohydrate (%)	Moisture (%)	Protein (mg/L)
KORANGI FISH HARBOR					
	B. orientalis	5.98 ± 0.799 (4.88–7.1)	3.817 ± 5.586 (2.802–4.854)	69.910 ± 1.713 (67.749–71.983)	35.480 ± 127.135 (32.852–41.920)
	P. javanicus	6.192 ± 8.383 (3.66–8.76)	4.575 ± 1.119 (3.558–5.967)	71.806 ± 1.912 (68.668–73.562)	36.466 ± 456.809 (24.803–41.092)
	C. quardilintus	8.56 ± 6.308 (0.02–16.96)	4.062 ± 4.048 (3.558–4.728)	75.973 ± 3.389 (71.626–79.865)	41.415 ± 229.348 (29.5118–50.065)
	C. arel	19.044 ± 9.017 (11.06–34)	4.255 ± 1.560 (2.352–6.125)	74.347 ± 2.864 (71.827–78.758)	178.274 ± 145.805 (134.657–211.601)
KETI BANDAR FISH HARBOR					
	B. orientalis	11.124 ± 14.398 (1.8–36.42)	5.256 ± 1.194 (3.562–6.458)	69.252 ± 17.605 (55.896–99.996)	465.391 ± 127.135 (328.658–670.806)
	P. javanicus	18.188 ± 3.320 (5.14–12.74)	5.212 ± 4.978 (3.215–8.256)	70.171 ± 1.745 (67.621–72.407)	601.369 ± 456.809 (165.300–839.098)
	C. quardilintus	12.722 ± 8.705 (4.54–16.88)	3.823 ± 5.724 (3.274–4.937)	71.296 ± 1.628 (68.804–72.938)	(731.403 ± 390.130) 282.254–1126.127
KARACHI FISH HARBOR					
	D. commersonnii	7.613 ± 5.046 (3.06–13.04)	4.264 ± 3.991 (3.333–4.748)	74.27 ± 0.685 (73.536–74.889)	25.7239 ± 10.52131 (14.207–34.832)
	C. puncticeps	6.948 ± 4.229 (4.06–14.26)	3.155 ± 0.581 (2.358–3.951)	71.979 ± 1.027 (70.543–72.921)	25.688 ± 5.343 (18.761–30.321)
	B. orientalis	6.85 ± 1.185 (5.84–8.85)	2.940 ± 0.140 (2.752–3.146)	73.354 ± 7.754 (5.405–85.759)	91.890 ± 123.689 (7.720–308.953)
	C. quardilintus	10.432 ± 4.714 (6.9–17.84)	3.478 ± 0.747 (2.487–4.579)	72.584 ± 2.447 (70.036–76.031)	37.331 ± 31.922 (3.103–81.229)
	P. javanicus	7.684 ± 8.383107 (5.32–11.14)	5.227 ± 0.940 (4.003–6.233)	73.693 ± 1.027 (69.132–76.698)	112.012 ± 82.185 (10.118–209.661)
	P. elevatus	13.632 ± 16.906 (6.72–22.38)	3.872 ± 0.499 (3.198–4.521)	75.433 ± 4.909 (70.317–82.325)	414.956 ± 332.142 (356.374–1196.785)
	C. arel	29.64 ± 17.085 (15.56–51.58)	5.820 ± 0.478 (5.217–6.425)	69.232 ± 3.808 (64.919–74.961)	31.045 ± 35.946 (26.992–34.389)

PASNI FISH HARBOR				
C. quardilintus	10.248 ± 8.705 (3.52–25.16)	4.174 ± 4.165 (3.516–4.676)	75.293 ± 1.098 (74.097–77.006)	564.561 ± 229.348 (391.439–932.325)
C. arel	4.46 ± 0.913 (2.88–5.08)	5.200 ± 0.531 (4.481–5.821)	75.370 ± 1.151 (73.720–76.897)	407.607 ± 145.805 (242.570–615.584)
ORMARA FISH HARBOR				
C. punctieceps	65.448 ± 89.311 (14.84–224.4)	3.434 ± 0.307 (3.151–3.917)	70.271 ± 2.603 (65.979–72.289)	262.328 ± 200.726 (84.566–557.632)
C. arel	22.1 ± 4.352 (14.88–25.8)	4.62 ± 0.494 (4.013–5.218)	72.067 ± 1.335 (70.786–73.708)	193.311 ± 145.805 (112.808–248.974)
C. quadrilinatus	22.664 ± 10.081 (5.9–30.36)	3.533 ± 0.426 (2.816–3.929)	70.403 ± 1.606 (69.259–73.228)	490.168 ± 201.040 (282.464–820.096)
P. javanicus	28.272 ± 89.311 (3.66–32.56)	6.093 ± 0.343 (5.672–6.511)	73.175 ± 4.069 (68.618–76.767)	807.812 ± 322.038 (323.409–1145.027)

Table 3. ANOVA inter and intraspecific variation in the biochemical composition of sole fish species.

Site/Parameters	Species	F-value	P-value
CARBOHYDRATE			
Karachi,Keti,Korangi	*Euryglossa orientalis*	8.66	0.005*
Korangi,Keti,Karachi,Ormara	*Pseudorhombus javanicus*	1.23	0.333
Karachi,Keti,Ormara,Korangi,Pasni	*Cynoglossus quardilintus*	0.98	0.439
Karachi, Ormara	*Cynoglossus puncticeps*	0.90	0.371
Korangi,Khi,Ormara,Pasni	*Cynoglossus arel*	2.96	0.064*
Karachi/Karachi	*C.elevatus/D.commorsonii*	1.88	0.242
LIPID			
Karachi,Keti,Korangi	*Euryglossa orientails*	0.54	0.595
Karachi,Keti,Ormara,Korangi,Pasni	*Psedumorhambus javanicus*	53.14	0.000***
Karachi,Keti,Ormara,Korangi,Pasni	*Cynoglossus quadrilineatus*	2.99	0.044
Karachi, Ormara	*Cynoglossus puncticeps*	2.14	0.182
Korangi,Karachi,Ormara,Pasni	*Cynoglossus arel*	5.67	0.008*
Karachi/Karachi	*C.elevatus/D.commorsonii*	2.66	0.178
MOISTURE CONTENT			
Karachi,Keti,Korangi	*Euryglossa orientalis*	0.20	0.825
Korangi,Keti,Karachi,Ormara	*Pseudorhombus javanicus*	1.46	0.263
Karachi,Keti,Ormara,Korangi,Pasni	*Cynoglossus quardilintus*	6.60	0.001*
Karachi, Ormara	*Cynoglossus puncticeps*	2.09	0.187
Korangi,Khi,Ormara,Pasni	*Cynoglossus arel*	5.98	0.006*
Karachi/Karachi	*C.elevatus/D.commorsonii*	0.14	0.724
PROTEIN			
Karachi,Keti,Korangi	*Euryglossa orientalis*	26.01	0.000***
Korangi,Keti,Karachi,Ormara	*Pseudorhombus javanicus*	14.37	0.000***
Karachi,Keti,Ormara,Korangi,Pasni	*Cynoglossus quardilintus*	10.21	0.000***
Karachi, Ormara	*Cynoglossus puncticeps*	6.94	0.030
Korangi,Khi,Ormara,Pasni	*Cynoglossus arel*	18.59	0.000***
Karachi/Karachi	*C.elevatus/D.commorsonii*	22.74	0.009*

*=highly significant

Moisture Content

Highest Moisture content found in *P. elevates*, on the other hand, lowermost was in *C. punctieceps* within species (Table 1). Within sites highest moisture value was 75.973 % in *C. quardilintus* from Korangi fish harbor and lowest value was 69.252% in *Brachirus orientalis* from Keti Bandar fish harbor (Table 2).

Level of significance through statistical analysis one way anova showed very excessive significance difference in biochemical composition such as protein, lipid, moisture content and carbohydrates of seven sole fish species within species. Proximate composition showed highly significance difference. Normal distribution of sole fish

proximate composition shown graphically as an example of histograms Carbohydrate, moisture content lipid and protein.

DISCUSSION

The proximate nutrient concentration throughout all fish specimens showed variability among the different species. Fish is known as highly proteinous food which is consumed by populace. Because of fish quality, tastes and palatability a greater consumers eat it and also owing to its nutritional value (Adewoye *et al*., 2003).

Lipids are an essential component of the nutrition, from required parts of the protoplasm and differ between species (Parate, 2013; Sahar and Kanwal, 2019). Lipid is prime storage material in fish. However lipid content is additional energy present for forthcoming reproduction, and growth (Love, 1970; Adams, 1999; Tocher, 2003). Current study found the highest lipid concentration was observed in *Cynoglossus punctieceps* with 36.198% whereas lowest found in *Dagetichthys commersonnii* with 7.613% which compared to previous work with the highest value of 3.50% (Pradhan *et al*., 2015).

Presence of carbohydrates in aquatic environment is natural and convert predominant organic components into organic and can be transfer to other organic via biological activities (Sannigrahi *et al*., 2005; Myklestad and Børsheim, 2007; Khodse *et al*., 2010). Highest carbohydrates concentration was studied in *Pseudorhombus javanicus* with 5.277% and lowermost found in *C. punctieceps* with 3.2949% and it is compared to previous work their highest value was 9.22% (Udo and Arazu, 2012). Thus it is differ from our reported highest value it can be environmental factor or other biological factor.

Fish meat contains high water and low lipid as compared to chicken meat or beef (Nestel, 2000). Ranged of moisture content stuck between 71.1745% to 75.433% the highest moisture content reported from *P. elevates* 75.433% *C. punctieceps* 71.1745% and the least was 70.839% in *Brachirus orientalis* as they were compared to previous study which shows the similar value that was 75.16 % (Pradhan *et al*., 2015).

Fish is the highest source of Protein and low-priced which is in access to everyone (Louka *et al.*, 2004; Mohanty *et al*., 2019). Protein concentration was estimated from sole fishes within sites the highest reported value was 807.812 mg/ml (66.496%) in *P. javanicus* from Ormara fish harbor and lowest reported value was 25.688 mg/ml (2.572%) in *C. puncticeps* from Karachi fish harbor and compared to previous study where lowest value was19.76% (Udo and Arazu, 2012) which shows the big gap between low and highest value due to environmental factor.

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