Biochemical composition of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones from the Oman Sea and Caspian Sea

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Abstract

The present work was conducted to investigate the chemical composition (amino acid, mineral content, and lipid profile) of bones from three different Iranian pelagic fish species. The biochemical composition of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones captured from the Iranian Ocean (the Oman Sea and Caspian Sea) were determined. The analysis of amino acids and fatty acid profiles was done by HPLC and GC and also analysis of minerals was done by means of flame atomic emission spectrophotometry. The least amount of mono unsaturated fatty acids was observed in *C. engrauliformis* ($p<0.05$). The highest contents of polyunsaturated fatty acids and n3/n6 ratio were observed in bones of *C. engrauliformis*. High levels of Ca, Fe, P, Zn and Cu minerals were measured in *S. gibbosa* and *C. engrauliformis*, respectively. The highest values of basic amino acid and sulfur containing amino acids were measured in *S. gibbosa* and *C. engrauliformis*, respectively. The chemical index did not reveal lack of any essential amino acids in three studied fish varieties compared to reference protein. On the basis of result, the best bone composition was observed in *S. gibbosa*. Therefore, the use of bone powders varieties of these fish could be useful in food industries.

Keywords: Biochemical composition, Bone, *Sardinella gibbosa*, *Clupeonella engrauliformis*, *Stolephorus indicus*

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Introduction

Fishes are among the chief sources of animal protein, minerals, vitamins, polyunsaturated fatty acids and required nutrients for human consumption (Zuraini *et al*., 2006). Fish and fish products including fish meal and fish sauces comprise important sources of income worldwide (Sathivel *et al*., 2002). There are significant amounts of micro and macro-elements in fish such as calcium, phosphorous, selenium and manganese. Fish lipids are rich in long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Zhao *et al*., 2010; Ayas, 2012). It should be notified that the fatty acids, cholesterol and mineral composition of fish species vary based on the region of fishery, sex and age of the fish (Miniadis-Meimaroglou *et al*., 2007).

There has been an increase in production of fish, crustaceans, molluscs and other aquatic animals, giving rise to 97.168 million tonnes in 2013 (FAO, 2015). Over 50% of total worldwide fish production comes from marine sources, but approximately 70% of this amount is used for processing purposes prior to their final sale (FAO, 2011). A total of 20-80% of fish body, including fins, bones, heads, skin and viscera are discarded at different levels of processing that accounts for 20 million tonnes of waste, equivalent to 25% of total marine capture worldwide (AMEC, 2003). The fish wastes comprise considerable amounts of protein rich compounds that could be further processed and used as fish meals, supplements and other valuable products. Fish bones accounting for 10-15% of fish body weight is the major waste generated in food processing (Toppe *et al*., 2006). Earlier published papers confirm the presence of minerals, carbohydrate, lipids and proteins in fish bones, but information regarding fish bone composition is limited (Jones *et al*., 1999). Hence, fish bones and cut offs could be used as alternative raw materials for many supplementary products and as aquaculture food (Toppe *et al*., 2006).

Kilka (*Clupeonella* sp.) and Anchovy (*Stolephorus* sp.*)* fishing is one of the major sources of income of fishermen in Iran and anchovy sprat constitutes about 80–90% of the total kilka catches in the Caspian Sea. Moreover, kilka and anchovy are processed for fish oil, fish sauce and fishmeal production or they are directly used as food. Despite the demands for high quality fish meals, the quality of meals is often altered by poor handling. The recent collapse of kilka fisheries has affected the economy and nutrition of local people near the Caspian Sea adversely. Thus, finding an alternative way instead of fish meal production, in order to produce value added and higher quality products fulfilling market demand would be profitable. The aim of the present work was to investigate the chemical composition (amino acid, mineral content, and lipid

profile) of bones from three different pelagic fish species, including gold stripe sardine (*Sardinella gibbosa*), Anchovy kilka (*Clupeonella engrauliformis*) and Indian anchovy (*Stolephorus indicus*) caught from the Oman Sea and the Caspian Sea.

Material and methods

A total of 150 gold stripe sardine (*Sardinella gibbosa*), and 300 Indian Anchovy (*Stolephorus indicus*), collected from the fishing area of the Jask port (north coast of the Oman sea) and 150 Anchovy kilka (*Clupeonella engrauliformis*), collected from Babolsar fishing port (south coast of the Caspian Sea) in 2009 were used in the present study. The mentioned ports are famous commercial fish catching sites in the north and south of Iran. Fishing was carried out randomly in September 2012 with the support of professional local fishermen. The samples were stored at – 20ºC for further use.

The frozen specimens were defrosted by overnight incubation at 4°C. All the fish specimens were gutted, filleted and their gills were removed. After removing of the remaining meat and connective tissues, the bones were dipped in 1% trypsin solution (pH=6.9) for 3 hours at room temperature (Malde *et al*., 2010). The clean bones were stored at -20°C until use. All chemicals used were of analytical grade and analyses were carried out in triplicates each having at least 300 fish specimens. Data are given as mean±standard deviation.

The proximate analysis of bone contents of three fish species was carried out according to AOAC procedures. Crude protein (N×6. 25) was estimated by the Kjeldahl method (Foss, Germany). The moisture content was measured after drying for 4 h at 105°C, and after combustion of specimens for 16 h at 550°C the ash was determined (AOAC, 2012). The total crude fat was also extracted and measured by Bligh and Dyer (1959) method.

The Fe, Zn, Cu, Mg, Ca, Na, P, Cr, I, Cl, F and K contents of fish bones were determined after the destruction of organic contents of the samples by dry-ashing in a furnace oven, (Lilienthal L3/P, Bremen, Germany). To prevent mineral loss, the samples were kept at 90 – 250°C (ramp time 2 h, hold time 2 h), 525°C (ramp time 5 h, hold time 7 h) and 525 – 100°C (ramp time 2 h). After cooling, the samples were digested using 20 ml 65% nitric acid and dried in an oven. The digested samples were again placed in a furnace at 525 °C for 1 hour and the resulting white ash was again digested with 2 ml 65% nitric acid with 0.5 mL 30% H_2O_2 solution (Supra pure, Merck). The final volume was made up to 50 ml using distilled deionized water. Na and K measurements were demonstrated by flame atomic emission spectrophotometry while P analysis was carried out by visible-ultraviolet

spectrophotometry using the ammonium vanadate molybdate colorimetric (AOAC, 2012). The P absorbance values were read in a Hitachi-2000 double beam molecular spectrophotometer (Tokyo, Japan). All other Minerals were determined using a flame atomic absorption spectrophotometer (Perkin-Elmer AA spectrophotometer 3100, USA) with an air acetylene flame (Martínez-Valverde *et al*., 2000).

Total lipids were extracted with methanol: chloroform (2:1) method while fatty acids were obtained with 12% boron trifluoride (BF_3) in methanol and fatty acid methyl esters (FAMEs) were prepared by n-hexane (Moss *et al*., 1974). FAMEs were subsequently analysed by capillary gas chromatography SGE BPX70 (Philips, Netherland). The conditions were as follows; detector temperature at 280ºC, injector temperature at 240ºC, helium speed at 1.0 mL/min, and oven temperature ranged from 180 to 250ºC. Thrombogenisity Index (TI) was calculated according to (Ulbricht and Southgate, 1991).

To extract total amino acids, 20 mg of sample was hydrolyzed by 6 N chloric acid at 110°C for 20 hours. Amino acid separation was performed by HPLC (Knauer, Germany) using pre-column ortho-phtalaldehyde (OPA) and 7-fluoro-4-nitrobenzo-2-oxa-1, 3-diazol (NBD-F) in a Knauer C18 column (3.9 mm×150 mm). Fluorescent detector was set at 250 and 395 nm. Cysteine was determined by 4 N NaOH digestions at 110°C for 24h. The tryptophan content was determined by the colorimetric method of (Witte *et al*., 2002). All determinations were performed in triplicate.

Essential amino acid scores (AS) were calculated according to reference amino acid requirements for adults (Witte *et al*., 2002) and given by the Eq. (1).

AS = (Sample amino acid/ reference amino acid) × 100 (Eq. 1)

The total essential amino acid (Σ EAA) to the total amino acid (Σ AA) ratio, i.e. (Σ EAA/ Σ AA); total sulfur amino acid (Σ SAA) and total aromatic amino acid (Σ ArAA) were determined. The Leu/Ile ratios were calculated while the predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer *et al*. (1974), i.e. P-PER= -0.468 + 0.454 (Leu) - 0.105 (Tyr).

Differences between mean values were computed using one way analysis of variance (ANOVA). Duncan's multiple range tests was also carried out to conduct *post hoc* comparisons between pairs of treatments. Statistically significant differences were demonstrated at $p<0.05$. All calculations were performed by SPSS 16 software.

Results

Proximate composition of *S. indicus*, *S. gibbosa*, and *C. engrauliformis* bones are presented in Table 1. Moisture contents of bones ranged from 6.63±0.29 g/100g in *C. engrauliformis*

to 10.17±0.45 g/100g in *S. indicus* (p<0.05). The highest crude lipid and ash contents among studied fish species were seen in *C. engrauliformis* and showed significant differences when compared to *S. gibbosa* and *S. indicus* (p<0.05).The ash/protein ratio showed the highest value in *C. engrauliformis* followed by that in *S. gibbosa* and *S. indicus.*

The mineral composition of *S. indicus, S. gibbosa,* and *C. engrauliformis* bones are described in Table 2. The highest level of calcium, iron, phosphorous and zinc were found in *S. gibbosa* whereas *C. engrauliformis* had the lowest amount of these minerals. The contents of Mg did not present significant differences among the three fish species. The highest amount of copper and chromium was observed in *C. engrauliformis* and other species did not exhibit significant differences in these minerals (p<0.05). The minimum content of sodium and chlorine were measured in *C. engrauliformis* bones and *S. gibbosa* showed highest potassium, fluorine and iodine contents.

The fatty acid composition of bones in three fish species is listed in Table 3. The analysis of *S. indicus, S. gibbosa*, and *C. engrauliformis* bones did not show significant differences in myristic acid, palmitic acid, linolenic acid, eicosanoic acid, arachidonic acid and eicosapentaenoic acid contents (p>0.05). The total fatty acid contents constituted 26.07±0.36 g/100g in *C. engrauliformis* to 27.23±0.30 g/100g in *S. indicus* (Table 3).

Moreover, S. *indicus* exhibited the greatest amount of stearic acid and oleic acid (p<0.05). The level of docosahexaenoic acid showed significant differences among the three species and*S. Gibbosa* showed the maximum docosahexaenoic acid content (p<0.05). Saturated and monounsaturated fatty acid contents did not show any significant differences (p>0.05).The maximum n-3 polyunsaturated fatty acids were found in *S. gibbosa* fish species (p<0.05). Subsequently, maximum n-6 polyunsaturated fatty acids were found in *S. Indicus* (p<0.05).

Amino acid composition of the examined fish species is given in Table 4. The results showed that unlike other amino acids, isoleucine, cysteine, tyrosine and valine contents of *S. indicus, S. gibbosa*, and *C. engrauliformis* bones didn't show any significant differences (p>0.05). The highest content of essential amino acids was observed in *S. gibbosa* bone.

Chemical index and protein efficiency ratios based on reference protein required by adults are presented in Table 5. Therefore, none of the three fish species showed lack of essential amino acids compared to reference amino acids and all chemical index values were more than one (CI>1).

Table 1: Proximate composition of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones (g/100g).

Proximate	*S. indicus*	*S. gibbosa*	*C. engrauliformis*
Moisture	10.17 ± 0.45[a]	7.03 ± 0.20[b]	6.63 ± 0.29[b]
Lipid	25.20 ± 1.40[a]	27.05 ± 1.00[a]	31.60 ± 1.22[b]
Protein	36.30 ± 1.23[a]	34.67 ± 0.65[a]	27.53 ± 1.85[b]
Ash	23.17 ± 1.04[a]	27.20 ± 0.96[b]	31.23 ± 1.25[c]
lipid free dry matter			
Protein	57.88[a]	53.77[b]	45.44[c]
Ash	36.95[a]	42.18[b]	51.55[c]
Ash/raw protein	0.64[a]	0.78[a]	1.13[b]

Values are expressed as mean ± SE of three replicates. Different letters within a row denote significant differences (p<0.05).

Table 2: Mineral composition of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones from lipid free dry matter.

Mineral	*S. indicus*	*S. gibbosa*	*C. engrauliformis*
Ca (g/kg)	176.33 ± 3.51[b]	206.67 ± 6.81[c]	148.67 ± 3.05[a]
P (g/kg)	82.67 ± 4.72[b]	92.67 ± 3.21[c]	76.33 ± 3.21[a]
Mg (g/kg)	2.63 ± 0.21[a]	3.07 ± 0.15[a]	2.10 ± 0.10[a]
Fe (mg/kg)	56.00 ± 2.64[b]	69.00 ± 2.64[c]	47.23 ± 1.53[a]
Zn (mg/kg)	146.33 ± 1.53[b]	195.00 ± 7.00[c]	138.33 ± 3.51[a]
Cu (mg/kg)	0.87 ± 0.58[a]	1.07 ± 0.06[a]	2.87 ± 0.15[b]
Cr (mg/kg)	5.80 ± 0.10[a]	6.73 ± 0.15[a]	9.17 ± 0.80[b]
Na (g/kg)	6.53 ± 0.30[b]	7.67 ± 0.06[b]	2.67 ± 0.32[a]
K (mg/kg)	4.53 ± 0.11[a]	6.37 ± 0.15[b]	3.50 ± 0.20[a]
I (mg/kg)	2.60 ± 0.20[a]	3.67 ± 0.11[b]	3.23 ± 0.30[ab]
Cl (g/kg)	4.20 ± 0.10[b]	4.87 ± 0.06[b]	2.70 ± 0.10[a]
F (g/kg)	0.07 ± 0.01[a]	0.40 ± 0.52[b]	0.03 ± 0.01[a]

Values are expressed as mean ± SE of three replicates. Different letters within a row denote significant differences (p<0.05).

Table 3: Fatty acid composition of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones (g/100g).

Fatty acids	*S. indicus*	*S. gibbosa*	*C. engrauliformis*
14:00 Myristic acid	2.77 ± 0.11[a]	2.70 ± 0.17[a]	2.43 ± 0.32[a]
16:00 Palmitic acid	18.80 ± 0.20[a]	19.70 ± 0.44[a]	18.80 ± 0.56[a]
18:00 Stearic acid	5.67 ± 0.42[a]	4.17 ± 0.40[b]	4.84 ± 0.46a[b]
Σ SFA	27.23 ± 0.30[a]	26.57 ± 0.90[a]	26.07 ± 0.36[a]
16:1 n-7 Palmitoleic acid	3.93 ± 0.15[a]	2.17 ± 0.17[b]	3.38 ± 0.85[a]
18:1 n-9 Oleic acid	19.90 ± 1.10[a]	19.40 ± 0.40[b]	18.30 ± 0.44[b]
20:1 n-9 Eicosanoic acid	1.23 ± 0.15[a]	0.83 ± 0.06[a]	1.13 ± 0.07[a]
Σ MUFA	25.07 ± 1.13[a]	24.47 ± 0.27[a]	22.81 ± 1.23[a]
18:3 n-3 Linolenic acid	1.03 ± 0.15[a]	0.77 ± 0.06[a]	0.94 ± 0.06[a]
20:5n-3 Eicosapentaenoic acid	5.90 ± 0.20[a]	6.60 ± 0.46[a]	6.70 ± 0.17[a]
22:6 n-3 Docosahexaenoic acid	20.67 ± 0.61[a]	28.27 ± 0.30[c]	24.23 ± 0.45[b]
Σ n-3 PUFA	27.60 ± 0.66[a]	35.63 ± 0.57[c]	31.87 ± 0.38[b]
18:2 n-6 Linoleic acid	4.53 ± 0.30[a]	3.67 ± 0.42[a]	3.70 ± 0.26[a]
20:4 n-6 Arachidonic acid	1.03 ± 0.15[a]	0.80 ± 0.10[a]	0.87 ± 0.11[a]
Σ n-6 PUFA	5.57 ± 0.15[a]	4.47 ± 0.32[b]	4.57 ± 0.32[b]
Σ n-3/n-6 PUFA	0.20 ± 0.008[a]	0.12 ± 0.01[a]	0.14 ± 0.009[a]
EPA+DHA	26.57 ± 0.50[a]	34.87 ± 0.59[c]	30.93 ± 0.32[b]
TI	0.26 ± 0.00[a]	0.20 ± 0.00[a]	0.18 ± 0.08[a]

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, AI: atherogenic index, TI: thrombogenic index. Values are expressed as mean ± SE of three replicates. Different letters within a row denote significant differences (p<0.05).

Table 4: Amino acid profile of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones.

Amino acids	*S. indicus*	*S. gibbosa*	*C. engrauliformis*
Arg	7.41± 0.23^{b}	8.61 ± 0.28^{a}	7.83 ± 0.06^{b}
His	1.83 ± 0.06^{b}	2.40 ± 0.03^{a}	2.35 ± 0.02^{a}
Ile	2.74 ± 0.08^{a}	2.55 ± 0.05^{a}	2.30 ± 0.10^{a}
Leu	5.53 ± 0.02^{b}	4.78 ± 0.07^{a}	5.44 ± 0.11^{b}
Lys	4.54 ± 0.02^{b}	5.53 ± 0.02^{a}	5.44 ± 0.01^{a}
Met	3.51 ± 0.02^{a}	3.47 ± 0.11^{a}	3.64 ± 0.04^{a}
Cys	1.03 ± 0.05^{a}	1.04 ± 0.06^{a}	1.77 ± 0.15^{a}
Phe	4.38 ± 0.05^{b}	3.80 ± 0.08^{a}	3.24 ± 0.06^{c}
Tyr	1.43 ± 0.01^{a}	1.32 ± 0.01^{a}	1.81 ± 0.02^{a}
Thr	3.98 ± 0.01^{a}	3.53 ± 0.03^{ab}	3.40 ± 0.05^{b}
Try	0.42 ± 0.01^{b}	0.63 ± 0.02^{a}	0.68 ± 0.03^{a}
Val	3.54 ± 0.05^{a}	3.74 ± 0.16^{a}	3.53 ± 0.05^{a}
Σ EAA	36.67 ± 0.28^{b}	38.20 ± 0.66^{a}	37.95 ± 0.36^{a}
Ala	7.04 ± 0.10^{b}	6.83 ± 0.11^{a}	6.41 ± 0.32^{a}
Asp	6.80 ± 0.01^{b}	7.10 ± 0.06^{ab}	7.87 ± 0.35^{a}
Glu	10.33 ± 0.01^{a}	10.47 ± 0.03^{ab}	10.98 ± 0.15^{b}
Gly	17.20 ± 0.31^{b}	16.50 ± 0.52^{a}	15.92 ± 0.62^{a}
Pro	7.20 ± 0.20^{b}	6.42 ± 0.02^{a}	7.33 ± 0.15^{b}
Hyp	1.88 ± 0.03^{b}	2.57 ± 0.02^{a}	2.79 ± 0.02^{a}
Ser	5.64 ± 0.11^{a}	5.92 ± 0.02^{a}	5.23 ± 0.15^{b}
Σ NEAA	56.41 ± 0.42^{b}	55.81 ± 0.34^{a}	56.52 ± 0.60^{b}
Σ AA	96.43 ± 0.33^{b}	97.21 ± 0.33^{a}	97.96 ± 0.52^{a}
Σ BAA	13.79 ± 0.29^{b}	16.54 ± 0.28^{a}	15.63 ± 0.08^{c}
Σ AAA	17.13 ± 0.03^{c}	17.57 ± 0.16^{b}	18.85 ± 0.10^{a}
ΣSAA	4.86 ± 0.07^{a}	4.84 ± 0.12^{a}	5.40 ± 0.15^{b}
Σ ArAA	6.23 ± 0.09^{a}	5.75 ± 0.02^{b}	5.73 ± 0.05^{b}
Leu/Ile ratio	2.01 ± 0.06^{ab}	1.87 ± 0.02^{a}	2.40 ± 0.06^{b}

EAA: essential amino acids, NEAA: non-essential amino acids, BAA: basic amino acids, AAA: acidic amino acids, SAA: sulphur amino acids, ArAA: aromatic amino acids, P-PER: predicted protein efficiency ratio. Values are expressed as mean±SE of three replicates. Different letters within a row denote significant differences (p<0.05).

Table 5: Chemical index (CI) and protein efficiency of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones.

Amino acid	Reference protein*	*S. indicus*	*S. gibbosa*	*C. engrauliformis*
Histidine	1.6	1.14	1.50	1.47
Isoleucine	1.3	2.11	1.97	1.77
Leucine	1.9	2.90	2.52	2.91
Lysine	1.6	2.80	3.45	3.40
Methionine + Cysteine	1.7	2.85	2.84	3.18
Phenylalanine+Tyrosine	1.9	3.05	2.70	2.65
Threonine	0.9	4.42	3.92	3.77
Valine	1.3	2.72	2.87	2.57
Protein efficiency		2.32	2.03	2.36
Protein efficiency		2.06	2.10	2.17
Protein efficiency		2.35	2.40	2.35

* Essential amino acids required for an human adult (WHO/FAO, 1985).

Discussion

The chemical composition of fish bones, including protein, lipid and ash contents varies substantially in different species. For instance, fatty fish such as salmon tends to accumulate higher lipids in bones compared to lean fishes like cod that predominantly store lipids in liver (Toppe *et al*., 2006). The highest crude lipid and ash contents among studied fish species have been observed in *C. engrauliformis*. This lipid content was in concordance with 38.2 and 36 g/100g of lipids reported in salmon and trout fish (Toppe *et al*., 2006). Consequently, crude protein estimates with the highest measures in *S. indicus* and lowest in *C. engrauliformis*.

Lipid free dry matter analysis showed significant difference of protein and ash contents among the three studied fish species. As it is shown in Table 1, the level of lipid free ash and proteins of *S. indicus* and *S. gibbosa* inversely correlated which was in concordance with earlier reports (Toppe *et al*., 2006). *S. indicus* exhibiting the greatest lipid free protein content showed the lowest amount of ash. There is less information regarding the chemical composition of fish bones, but the studies done show the similar values of ash but higher protein and lipid contents in blue whiting fish (FAO, 1973).

The ash/protein ratio showed the highest value in *C. engrauliformis* (1.13) followed by *S. gibbosa* (0.78) and *S. indicus* (0.64), which were close to the ratio of 0.78 scored by salmon and relatively lower than cod and horse mackerel (Toppe *et al*., 2006). However, the low ash/protein ratio in *S. gibbosa* and *S. indicus* could be attributed to their need for more elastic bones required for high physical activities.

Overall, the differences in biochemical composition of *C. engrauliformis* and *S. indicus* were more evident compared to that in *S. indicus* and *S. gibbosa*. Different habitats of *C. engrauliformis* and *S. indicus* emphasize biochemical differences of these two fish species. The Oman Sea contains a totally different environment including salt concentration, pH, and temperature compared to the Caspian Sea. On the other hand, despite the genetic differences and variable nutritional modes of *S. indicus* and *S. gibbosa*, they share a common habitat and environment in the Oman Sea.

The mineral composition of *S. indicus, S. gibbosa,* and *C. engrauliformis* bones are described in Table 2. The amount of Ca measured in our study was greater than that in tilapia bones (Alsmeyer, 1974). The highest amount of Cu and Cr was observed in *C. engrauliformis* and other species did not exhibit significant differences for these minerals ($p<0.05$).

Minerals hold high importance as the components responsible for the strength of bones. The present study revealed the presence of high Ca, P, Na and Cl in *S. gibbosa* and *S. indicus*

compared to *C. engrauliformis* bones. Moreover, the highest contents of Fe, Zn and I were observed in *S. gibbosa*. The earlier published study by (Toppe *et al*., 2006) proved that the Ca, Mg and P contents are dependent on ash present in the bones. The salt concentration of the Oman Sea (36 to 38 ppt) is higher than that in the Caspian Sea (10 to 12 ppt), providing more minerals for the growth and development of fishes living in the Oman Sea. On the other hand, unlike *S. gibbosa* and *S. indicus* bones, Cu and Cr concentration were high in *C. engrauliformis* reflecting the entry of pollutants into the Caspian Sea which requires further studies.

The fatty acid composition of bones in three studied fish varieties is listed in Table 3. The level of C16:0 measured in our study ranging from 18.80 ± 0.20 g/100g to 19.70±0.44 g/100g was significantly higher than those measured in cod fish (8.7 g/100g) and mackerel (12.5 g/100g) (Toppe *et al*., 2006).

The n-3/n-6 ratio are a crucial factor in the nutritional value of fish; hence a diet high in n-3/n-6 ratio is preferable. Moreover, n-3/ n-6 ratio range from 0.5 to 3.8 in freshwater fish and from 4.7 to 14.4 in sea fishes (Kleimenov, 1971). The studied fish varieties showed significant n-3/n-6 ratios including 8.01±0.7 (*S. gibbosa*), 7±0.45 (*C. engrauliformis*) and 4.96±0.19 (*S. indicus*). Thrombogenic index reflecting the effect of fatty acids on heart health and thrombosis was not significant due to high ω3 fatty acid contents. This index represents the nutritive and healthy composition of studied fish bones.

Amino acid composition of the examined fish varieties is given in Table 4. Chemical index and protein efficiency ratios based on reference protein required by adults are presented in Table 5.

All three fish species scored chemical index values above one (CI>1) that represents their nutritive nature for adult use (Vignesh and Srinivasan, 2012). The high content of essential amino acids (±SD) was observed in *C. engrauliformis* bone (37.95±0.36) and *S. gibbosa* (38.2±0.66). The amino acid content of 33-39.4% has been reported by Abdullahi and Abolude from fresh water fish bones (Kleimenov, 1971). Although the amount of essential amino acids measured in the *S. indicus*, *S. gibbosa* and *C. engrauliformis* bones were lower than the essential amino acids present in reference protein (56.6%), they could be suitable for infants, children and adults, as the required essential amino acid/total amino acid (E/T) values of these age groups were reported as 26%, 39% and 11%, respectively (FAO/WHO/UNU, 1985). The previously reported biochemical analyses showed that excess dietary leucine can affect tryptophan-niacin metabolism adversely (Rao, 1973). Also Belavady and Rao (1979), stated that isoleucine counteracts with excess leucine

molecules. In addition, it has been proved that balanced Leu/ Ile ratio is more important than leucine intake (Abdullahi and Abolude, 2002). The amount of isoleucine was evidently lesser than leucine in fish species studied in this research.

During the past twenty years, the role of glutamine has been studied in numerous body organs (Jones *et al*., 1999). Glutamine is the most occurring free amino acid and constitutes 60% of total intercellular amino acids. Glutamine is involved in purine and pyrimidine synthesis, immune system and wound healing (Rao, 1973; Belavady and Rao, 1979). The level of glutamic acid was higher in *S. gibbosa* and *C. engrauliformis* bones compared to that in *S. indicus* bones. In another report glutamic acid (Glu) were revealed as the amino acids with the highest levels in all seasons in the (*Barbus grypus*) (Olgunoglu *et al*., 2011). Studies have shown that cereal based diets in developing countries lead to lysine deficiency in children as cereals contain only 2.6-3.8% lysine but a total of 7-10% lysine is found in animal sources (Chyun and Griminger, 1984; Deutz *et al*., 1992; Khalil and Khan, 1995). The level of lysine was found as 5.44±0.005 in *C. engrauliformis*, 5.53±0.02 in *S. gibbosa* and 4.54±0.02 in *S. indicus*, but this value was lower than the recommended value by FAO/WHO (Vignesh and Srinivasan, 2012). In addition, the studied fish bones showed sufficient methionine and valine levels, but isoleucine and cysteine levels were lower compared to reference protein. A previous study by Adeyeye (2009), showed higher level of aspartic and glutamic acid compared to other essential amino acid in *Claris anguillaris, Cynoglossus senegalensis* and *Oreochromis niloticus*.

Glycine comprises the highest amount of amino acids in *S. indicus, S. gibbosa* and *C. engrauliformis* bones. Collagen is one of the main constituents of fish bone and glycine is the highest amino acid content of the animal collagen (Pellett, 1996). This explains the high content of glycine observed in the present study.

The overall results obtained from the present study indicated the rich composition of *S. gibbosa*, *S. indicus* and *C. engrauliformis* bones in amino acids, minerals and fatty acids that makes them suitable as food supplements in food industries.

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