Nutritional evaluation of processing discards from tiger tooth croaker, *Otolithes ruber*

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Abstract Processing discards from tiger tooth croaker, such as head, viscera, and swim bladder, were analyzed for proximate, biochemical, fatty acid, and mineral composition. The proximate analysis showed high protein content (21.04%) in the swim bladder and high fat content (4.10%) and ash content (2.26%) in the head. The biochemical quality indices such as non-protein nitrogen, total volatile base, trimethylamine, free fatty acids, peroxide value, and thiobarbituric acid reactive substances in the viscera were higher than those in the head and swim bladder. Gas chromatography-mass spectrometry analysis of fatty acids composition showed high polyunsaturated fatty acids in the head (58.82%), followed by the viscera (45.80%) and the swim bladder (35.57%). The major available saturated fatty acid, i.e., palmitic acid, was higher in the swim bladder (30.49%). Mineral and heavy metals analyzed via inductively coupled plasma-optical emission spectrometer showed high calcium, sodium, and magnesium contents in the head region (346.80, 62.55, and 14.89 mg/kg respectively). Heavy metal levels were within the permissible limit.

Keywords: tiger tooth croaker, omega-3 fatty acid, biochemical quality index, fish processing discard, mineral

Introduction

The Indian fishing industry has assumed a great importance during the last couple of decades because of the ever increasing demand of fish in the local as well as international markets. During the year 2014–2015, Indian exports of marine products reached 1,051,243 tons and were valued at US\$ 5,511.12 million (1). There are 465 seafood processing plants along the Indian coast, with a total built-in capacity of 20,256 MT. Especially, the Kerala coastal region has 99 processing plants, with a total capacity of 3,006 MT (1). Among the coastal states, Kerala contributes 5.76 lakh tons to the marine fish landing in India (2). Globally, the annual discards from fisheries, including by-products, is approximately 20 million tons per year (3). All over the world, more than 60% (w/w) of raw materials, which include frames, fins, head, skin, trimmings, viscera, and roes of fish are discarded as processing waste by the fish processing industries (4). In India, industrial fish processing generates 302,750 tons of discards (1). The waste generated in the fish processing industries not only causes environmental pollution (bad odor and proliferation of insects and pests) but also leads to the loss of valuable components such as proteins, lipids, and minerals. These discards, which contain a substantial amount of nutrients, are widely used for the production

of low-value by-products such as fertilizers, animal feed ingredients, and fish meal. Several biotechniques have been developed for the conversion of low-value fish processing discards into high-value products such as essential polyunsaturated fatty acids (PUFA), physiologically important peptides, saccharides, and other bioactive compounds. These recoverable compounds would help improve human health and increase waste utilization (4).

The croaker, commonly known as “drummer” and “jew fish,” comes under the Sciaenidae family. It constitutes 10–12% of the demersal catch of India. It is a well-known edible marine fish found in the both east and west coast throughout the year. Croaker is a carnivorous species and its diet comprises a wide range of animals, such as small fish, crustaceans, mollusks, and polychaetes (5). In India, croaker is also one of the major raw materials for surimi production. Croakers alone contributes 1.62 lakh tons during 2014–15 marine fish landing (2). Mainly the muscle parts are used in surimi production and the remaining parts discarded as waste. Chemical characterization of croaker discards is important to realize the conversion of low-value processing discards into high-value by-products. Currently, the croaker processing discards are mainly used for the production of fish meal, fish silage, and fish manure. Since croaker discards are one of the important bio resources, they can be

used for the recovery of bioactive molecules that are utilized in food, nutraceuticals, healthcare, and pharmaceutical industries for improving the economic value of these processing discards.

Numerous studies have investigated the omega-3 fatty acids in fish. This PUFA has several beneficial effects on human health, such as neural and visual development. Moreover, omega-3 fatty acids help in the prevention of life-threatening diseases such as hypertension, cardiovascular diseases, cancer, multiple sclerosis, diabetes, Alzheimer's disease, depression, cystic fibrosis, arthritis, schizophrenia, asthma, and attention deficit disorder (6). This study aims to profile the nutritional composition of processing discards from tiger tooth croaker in order to reveal the potency of fish-processing waste as secondary raw material for high-value products.

Materials and Methods

Raw material The head, viscera, and swim bladder of tiger tooth croaker (*Otolithes ruber*) were procured from the local fish market, Cochin, Kerala, India and brought to the laboratory in iced condition ($4\pm1^\circ\text{C}$) in an insulated container. The samples were washed in potable water and stored at 20°C . For each analysis, the samples were homogenized in a blender to achieve the homogeneity.

Chemicals Fatty acid methyl esters standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in this investigation were analytical grade.

Proximate composition of tiger tooth croaker discards Moisture, protein, lipid, and ash contents of croaker discards were determined using the Association of Official Analytical Chemists (AOAC) method (7). Moisture content was analyzed by hot air oven method. The total nitrogen content was determined using the Kjeldahl method to obtain the protein and expressed as % of protein on wet-weight basis. Total fat content was estimated using the petroleum ether extraction method. The ash content was determined using moisture-free samples by incinerating in a muffle furnace and expressed as % of ash on wet-weight basis.

Biochemical indices of tiger tooth croaker discards Non-protein nitrogen (NPN) content was estimated according to the AOAC method (7). The NPN values were expressed in mgN/100g. Total volatile base nitrogen (TVB-N) and trimethylamine (TMA) contents were determined using the Conway micro diffusion method (8) and values were expressed in mgN/100g. Free fatty acids (FFA) were estimated using the AOAC method (7) and expressed in oleic acid percentage. Peroxide value (PV) was determined by iron-based spectrophotometric method described by Shantha and Decker (9). PV value was expressed in meqO₂/kg of fat. Secondary lipid oxidation products were measured using thiobarbituric acid reactive substances (TBARS) value by the method of Raghavan and Hultin (10) and the

value was expressed in mg malonaldehyde/kg of sample.

Analysis of fatty acid profiles of tiger tooth croaker discards Lipids were extracted from the samples using the method described by Folch *et al.* (11). Fatty acid methyl esters (FAMES) were prepared according to Metcalfe *et al.* (12). Accurately 150 mg of oil was mixed with 6 mL of methanolic NaOH in a flask and refluxed. After the disappearance of fat globules, 7 mL BF₃ methanol was added to the mixture and boiled for 2 min. Then 15 mL of saturated NaCl was added and mixed vigorously for 15 s. From the reacted mixture, the fatty acid was extracted using 30 mL of petroleum ether. The extracted fatty acid was washed with water and dried by filtering through anhydrous Na₂SO₄. The excess solvent was evaporated under the stream of nitrogen in a water bath (TW20; Julabo, Seelbach, Germany).

FAMES were volatilized before conducting the gas chromatography–mass spectrometry (GC–MS) analysis. Fatty acids were separated using GC–MS (Autosystem XL-Gas Chromatograph-Turbomass Gold Mass Spectrophotometer, Perkin Elmer, Waltham, MA, USA) equipped with a 0.25 µm film thickness and 30 m×0.25 mm ID Carbowax capillary column (Cromlab S.A., J&W Scientific, Folsom, CA, USA). The carrier gas used in this analysis was helium. The injector and detector temperatures were set at 250°C . Split mode (1:15) was chosen for the injection of sample. The initial column temperature was set at 50°C for 2 min, and the final temperature 230°C was achieved by increasing the temperature at the rate of 10°C per minute. FAMES were separated at a constant pressure of 23.1 kPa and were identified by comparing the retention time with those for fatty acids standards. The obtained results were processed using the Star WS software package, and individual fatty acids were expressed as a percentage of total fatty acids.

Minerals and heavy metal analysis of tiger tooth croaker discards

Mineral composition of croaker waste and the heavy metals present in it were determined using inductivity coupled plasma–optical emission spectrometer (ICP-OES), according to the method described by Dolan and Capar (13). Croaker wastes were digested in a microwave-assisted extraction system, Milestone START D (Milestone Srl., Sorisole BG, Italy), equipped with easy-CONTROL software and 1000/10S high pressure segmented (HPR) rotor. Each sample weighed 1g and was transferred to acid-cleaned Teflon vessels. Then, 8 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added to the sample. The samples were then exposed to microwave digestion for 40 min; the operating conditions are listed in Table 1. The digested samples were diluted with ultrapure water in a 50 mL volumetric flasks prior to elemental profiling.

The digested samples were quantitatively analyzed for Aluminium (Al), Boron (B), Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Cadmium (Cd), Chromium (Cr), and Lead (Pb) using an ICP-OES (iCAP 6300 Duo; Thermo Fisher Scientific, Waltham, MA, USA) with dual

Table 1. Microwave digestion conditions in Milestone START D¹⁾ and Experimental conditions for elemental analysis using ICP-OES

Microwave digestion conditions	
Pressure	400 psi (max.)
Power	1200 W
Temperature	Step I: Ramp to 150°C over 30 min
	Step II: Hold at 150°C for 10 min
	Step III: Allow to cool to room temperature over 1 h
Experimental conditions for ICP-OES	
RF power	1150 W
Optics temperature	38°C
Camera temperature	44°C
Nebulizer	MiraMist
Spray chamber	Cyclonic Chamber
Main Argon flow rate	15 L min ⁻¹
Auxiliary Argon flow rate	0.5 L min ⁻¹
Nebulizer Gas Flow	0.5 L min ⁻¹
Maximum integration time	30 s

¹⁾Temperature and pressure sensors were used to monitor digestion conditions and to prevent over-pressurization of vessels.

configuration (axial and radial) and the iTEVA (version 2.8.0.97) operational software. The calibration solutions were prepared from the ICP multi-element standard solution (Certipur; Merck, Darmstadt, Germany). Yttrium was used as the internal standard.

Statistical analysis All analyses were carried out in triplicates, and the results are expressed as average mean±standard deviation. One-way analysis of variance was used to analyze the data, and the significant difference at $p<0.05$ between the means of triplicates were determined by the Duncans multiple range test using SPSS software (Version 20, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Physical characteristics and yield of the tiger tooth croaker Physical characteristics and yield of the tiger tooth croaker are listed in Table 2. The average total length and weight of the tiger tooth croaker were 19.06±2.71 cm and 59.40±3.46 g, respectively. Out of the total length, the head portion occupies 27.5 % (5.24±1.54 cm). In terms of biomass, the processing waste mainly comprised the head, which accounted for 32.04% of the total biomass. The differences in the percentage are dependent on the size, season, and feeding habits of the fish. Solid waste of fish, such as the head, skin, viscera, bone, frame, liver, gonad, and gut constitutes 20–60% of the total initial raw material. Dekkers *et al.* (4) reported that only 40% of original weight is edible fish meat. Another study showed that only 25% of the fish is utilized for processing and the remaining 75% of the fish is used as a by-product during fish processing (14).

Proximate composition of tiger tooth croaker discards The

Table 2. Physical characteristics and yield of tiger tooth croaker

Physical characteristics	Otolithes ruber
Average Total length (cm)	19.06±2.71 ¹⁾
Average Head length (cm)	5.24±1.54
Average weight (g)	59.40±3.46
Edible Portion (%)	58.29±1.39
Head waste (%)	32.04±2.35
Viscera (%)	4.60±0.83
Swim bladder (%)	3.04±1.15

¹⁾Values are mean±SD, $n=3$.

Table 3. Proximate composition of the processing discards of tiger tooth croaker

Proximate composition	Head	Viscera	Swim bladder
Moisture	75.87±1.11 ^{a1)}	81.15±1.07 ^b	73.75±1.24 ^a
Protein	15.20±1.09 ^a	13.16±1.00 ^b	21.04±0.92 ^c
Fat	4.10±0.51 ^a	2.47±0.45 ^b	2.51±0.53 ^b
Ash	2.26±0.93 ^a	1.19±0.52 ^{ab}	0.70±0.24 ^b

¹⁾Values are reported as mean±standard deviation for $n=3$. The superscript letters indicate the significant differences between the discards ($p<0.05$).

proximate composition of tiger tooth croaker discards, viz., moisture, protein, fat and ash contents are given in Table 3 and the values are expressed in % of wet-weight basis. Evaluating proximate composition is the most important aspect for determining the nutritional status of fish processing discards. Proximate compositions of different fish and shellfish have been studied by different researchers (15,16). However, the data available on the proximate composition of processing discards is limited. The viscera of croaker contain high moisture content (81.15%) and there is no significant difference between head and swim bladder ($p>0.05$). In croaker discards, the fat content was high in the head region (4.10%) whereas the moisture content was less. The head of a fish contains less amount of muscle and numerous bones and lipid-associated membranes compared to the other body parts. This could be the reason for the high fat content and low moisture content in the discards of the croaker head. There is no significant difference between the fat content in the viscera and the swim bladder. Nazeer and Kumar (17) reported that croaker viscera contains 81.2% moisture, 13.1% protein, 4.3% lipid, and 1.4% ash. Protein content was high in the swim bladder (21.04%), followed by the head (15.2%). Generally, swim bladder is known for high collagen content. Thus, protein content is higher in the swim bladder than in the head and the viscera. Tiger tooth croaker is a carnivore and feeds on small fish, prawns, crabs, squilla, *Acetes* sp, molluscs shells, and squids (5). This might be the reason for the high protein content in the viscera. Ash content is high in the head region (2.26%) probably because of the presence of numerous bones.

Biochemical quality indices of tiger tooth croaker discards Fish and fishery products are highly perishable commodities. Their quality deteriorates mainly because of the chemical changes in the

Table 4. Biochemical indices of the processing discards of tiger tooth croaker

Biochemical indices	Head	Viscera	Swim bladder
NPN ¹⁾ (mgN/100 g)	340.02±3.31 ²⁾	382.62±3.02 ^b	371.12±3.51 ^c
TMA (mgN/100 g)	0.84±0.13 ^a	3.55±0.14 ^b	1.98±0.11 ^c
TVB-N (mgN/100 g)	15.70±1.2 ^a	23.16±1.21 ^b	19.79±0.94 ^c
FFA (oleic acid %)	0.31±0.11 ^a	0.92±0.09 ^b	0.22±0.03 ^a
PV (meqO ₂ /kg fat)	0.87±0.13 ^a	1.91±0.44 ^b	0.94±0.32 ^a
TBARS (mg malonaldehyde/kg)	0.50±0.1 ^a	0.69±0.06 ^b	0.35±0.11 ^c

¹⁾NPN, non-protein nitrogen; TVB-N, total volatile base nitrogen; TMA, trimethylamine; FFA, free fatty acids; PV, peroxide value; TBARS, thiobarbituric acid reactive substances

²⁾Values are reported as mean±standard deviation for *n*=3. The superscript letters indicate the significant differences between the discards (*p*<0.05)

major constituents, such as proteins and lipids. Because the changes in biochemical constituents are good indicators of the quality of the fish, the chemical quality indices will help optimize the processing and storage conditions of the fish and fishery products. Biochemical quality indices of tiger tooth croaker discards, viz., NPN, TVB-N, TMA, FFA, PV, and TBARS are given in Table 4. NPN compounds are low-molecular-weight, water-soluble compounds, which constitute 9–18% of the total nitrogen in teleosts and 33–38% in elasmobranchs. The major components of the NPN fraction are volatile bases (ammonia and TMAO), guanidine compounds (phosphagens, creatine, and creatinine), free amino acids, nucleotides and purine bases, urea (in cartilaginous fish), imidazole dipeptides (carnosine, anserine, and balenine), and betaines (18). In tiger tooth croaker discards, high NPN is recorded in the viscera (382.62 mgN/100 g), followed by the swim bladder (371.12 mgN/100 g) and the head (340.02 mgN/100 g). NPN is formed by digestive enzymes and microbial degradation of nitrogenous compounds, such as protein and nucleotides. The higher NPN in the viscera possibly results from the action of digestive enzymes present in the intestinal tract and microbial activity. There was a significant difference in NPN content (*p*<0.05) in the head, viscera, and swim bladder of tiger tooth croaker (Table 4). Ninan and Zynudheen (19) reported that the NPN content in ice-stored, fresh tiger tooth croaker is 413 mgN/100 g.

TVB-N is an efficient spoilage indicator and it indicates the degree of protein breakdown into non-protein nitrogen compounds due to bacterial and enzymatic activity, which leads to smaller molecules such as amines (20). TMA can also be used as a spoilage indicator, and they are considered unreliable for use in fresh fish because they reflect the later stages of spoilage rather than freshness. The TVB-N and TMA contents were high in the viscera (23.16 and 3.55 mgN/100 g, respectively) (Table 4). This is possible because of the high enzymatic and microbial activity in the visceral region (20). The head region contains fewer amounts of TVB-N and TMA than that in the viscera because of fewer portion of muscles, lower endogenous enzyme activity, and lesser microbial load than those in the viscera and the swim bladder. Like NPN, TVB-N and TMA contents were

significantly different in the head, viscera, and swim bladder (Table 4). For fish, the maximum acceptable limit of TVB-N and TMA are 30–35 and 10–15 mgN/100 g, respectively (21). However, there is no set standard for the utilization of waste based on the TVB-N and TMA contents. In the present study, the TVB-N and TMA values in tiger tooth croaker discards were significantly low; this indicates the good quality of the samples.

In fish, during the post-mortem stage, an increase in the FFA level because of the enzymatic hydrolytic activity of esterified lipids is a common phenomenon. These FFA interact with myofibrillar proteins of fish and cause protein aggregation, which leads to a tough structure. The acceptable limit of FFA in fresh fish is 2–10% oleic acid, and it has a negative relationship with the freshness of the fish (22). The visceral discards of tiger tooth croaker had high FFA content of 0.92% oleic acid, and there is no significant difference (*p*<0.05) in the FFA content of head and swim bladder (Table 4). In the initial stage, the production of FFA is mainly due to endogenous enzymes, and in the later stages, microbial enzymes become predominant in the formation of FFA. Viscera is a place where the proportion of endogenous enzymes is the highest compared to that in other body parts. Viscera is a suitable harbor for a variety of microorganism. Thus, even in the initial stage, endogenous and microbial enzymes are surplus in the viscera and lead to the formation of higher amounts of FFA than the head and swim bladder. A previous study conducted on tiger tooth croaker had shown the FFA content in fresh fish is 4.30% oleic acid (23).

Primary lipid oxidation was measured using hydroperoxides and is expressed as peroxide value (PV). PV is an excellent quality indicator of primary lipid oxidation during the initial stage of spoilage, because in later stages, PV value decreases by the result of hydroperoxide decomposition (24). Among the three discards, the viscera exhibit high PV of 1.91 meqO₂/Kg. This might be because of the high lipase enzyme activity in the viscera than the head and the swim bladder. No significant difference was found between PV values in the head and swim bladder (*p*>0.05). A study conducted by Sharifian *et al.* (23) had shown high PV (11.69 meq O₂/Kg) in fresh croaker.

Although hydroperoxides are not considered as harmful substances that hamper the quality of food, they undergo further degradation and form smaller secondary oxidative products, viz., aldehydes, ketones, and small-chain carboxylic acids, hydrocarbons, lactones, and esters, which are responsible for the off-odour and off-flavour. Secondary oxidation was measured using the TBARS value, which was high in the viscera (0.69 mg malonaldehyde/kg), followed by head (0.50 mg malonaldehyde/kg) and the swim bladder (0.49 mg malonaldehyde/kg) (Table 4). The major components involved in the oxidation are lipids and oxygen. However, in our study, the TBARS value for the high lipid content in the head discards is lower than that in the visceral discards. Apart from lipids and oxygen, trace elements such as ferrous (Fe²⁺) and copper (Cu²⁺), along with various biochemical compounds, such as free amino acids, pigments, organic acids, and heme compounds, present in the muscle are involved in the

Table 5. Fatty acid composition (%) of tiger tooth croaker processing discards

Fatty acids	Head	Viscera	Swim bladder
C6:0	0.03±0.01 ^{a1)}	0.03±0.04 ^a	3.55±0.18 ^b
C8:0	0.03±0.01 ^a	0.03±0.01 ^a	0 ^b
C12:0	1.16±0.01 ^a	0.10±0.06 ^b	0.15±0.03 ^b
C13:0	0 ^a	0.03±0.04 ^b	0.06±0.01 ^c
C14:0	0.65±0.04 ^a	2.45±0.26 ^b	4.79±0.85 ^c
C15:0	0.18±0.01 ^a	0.61±0.01 ^b	0.92±0.01 ^c
C16:0	4.74±0.42 ^a	22.21±1.37 ^b	30.49±2.48 ^c
C17:0	0.18±0.04 ^a	0.92±0.03 ^b	1.08±0.02 ^c
C18:0	10.24±1.03 ^a	8.26±1.29 ^a	8.23±0.67 ^a
C20:0	0.43±0.02 ^a	1.22±0.14 ^b	0.84±0.12 ^c
C21:0	0.37±0.02 ^a	0.20±0.03 ^b	0.30±0.04 ^c
C22:0	0.85±0.04 ^a	0.77±0.45 ^a	1.23±0.04 ^a
C23:0	5.82±0.87 ^a	5.46±0.32 ^a	1.56±0.23 ^b
C24:0	3.33±1.07 ^a	2.21±0.15 ^a	0.83±0.27 ^b
C8:1	0 ^a	0.01±0.01 ^a	0.07±0.01 ^b
C14:1	0.19±0.02 ^a	0.92±0.01 ^b	0.09±0.01 ^c
C15:1	0.16±0.09 ^a	0 ^b	0.03±0.01 ^b
C16:1	2.41±0.82 ^a	0.03±0.01 ^b	0.04±0.01 ^b
C17:1	0.17±0.01 ^a	0.45±0.27 ^a	0.40±0.12 ^a
C18:1	6.60±0.27 ^a	5.80±0.58 ^a	7.26±0.66 ^b
C20:1	0.69±0.08 ^a	0.29±0.85 ^b	0.46±0.03 ^c
C24:1	1.99±0.05 ^a	1.93±0.47 ^a	1.73±0.59 ^a
C22:1n9	0.95±0.74 ^a	0.30±0.02 ^a	0.31±0.03 ^a
C20:2	0.25±0.03 ^a	0.58±0.33 ^a	0.56±0.34 ^a
C22:2	13.10±0.45 ^a	11.21±0.75 ^b	7.26±0.53 ^c
C18:3n3	0.50±0.04 ^a	0.46±0.08 ^a	0.53±0.03 ^a
C20:3n3	7.23±1.52 ^a	5.81±0.85 ^a	3.37±0.52 ^b
C20:5n3	13.60±1.81 ^a	8.35±0.86 ^b	7.37±0.48 ^b
C22:6 n3	20.82±1.45 ^a	17.39±2.35 ^{ab}	14.5±1.64 ^b
C18:2n6c	1.50±0.01 ^a	0.03±0.01 ^b	1.28±0.16 ^c
C18:2n6t	0 ^a	1.21±0.05 ^b	0 ^a
C18:3n6	0.12±0.01 ^a	0.12±0.02 ^a	0.17±0.02 ^c
C20:3 n6	1.50±0.05 ^a	0.45±0.08 ^b	0.33±0.16 ^b
C20:4n6	0.21±0.01 ^a	0.17±0.01 ^a	0.21±0.17 ^a
Sum of saturates	28.02	44.49	54.03
Sum of monoenes	13.16	9.71	10.40
Sum of polyenes	58.82	45.80	35.57
n-3	42.15	32.00	25.76
n-6	3.32	1.98	1.99
n-3/n-6	13.1	16.1	12.9
DHA/EPA	1.5	2.1	2

¹⁾Values are reported as mean±standard deviation for n=6. The superscript letters indicate the significant differences between the discards ($p<0.05$)

acceleration of lipid oxidation (25). The amount of secondary oxidative products in the visceral discards is higher than that in the head and swim bladder discards. Another study conducted on fresh tiger tooth croaker fish had shown a TBARS value of 0.83 mg malonaldehyde/kg (23).

Fatty acid profiles of tiger tooth croaker discards The fatty acid profiles of the processing discards of tiger tooth croaker are shown in Table 5. Individual fatty acids are expressed by the percentage of

Table 6. Mineral and heavy metal content (mg/kg) of tiger tooth croaker processing discards

Elements	Head	Viscera	Swim bladder
Al	0.67±0.05 ^{a1)}	0.72±0.08 ^{ab}	0.84±0.06 ^b
B	BDL ²⁾	BDL	BDL
Ca	346.75±8.1 ^a	37.0±1.65 ^b	9.73±0.37 ^c
Cu	0.11±0.01 ^a	0.14±0.03 ^a	0.07±0.005 ^b
Fe	1.40±0.06 ^a	2.66±0.35 ^b	1.18±0.009 ^a
K	83.57±2.57 ^a	58.28±1.58 ^b	30.97±1.05 ^c
Mg	14.89±0.39 ^a	8.66±0.22 ^b	3.05±0.10 ^c
Mn	0.07±0.001 ^a	0.06±0.01 ^a	0.03±0.01 ^b
Na	62.55±1.72 ^a	34.71±0.92 ^b	30.2±0.99 ^c
Ni	BDL	BDL	BDL
Cd	BDL ^a	0.003±0.00 ^b	BDL ^a
Cr	0.03±0.01 ^a	0.03±0.01 ^a	0.02±0.01 ^a
Pb	0.23±0.14 ^a	0.07±0.06 ^b	0.03±0.01 ^b

¹⁾Values are reported as mean±standard deviation for n=6. The superscript letters indicate the significant differences between the discards ($p<0.05$).

²⁾BDL, below the detection limit.

total fatty acids. There are 11 major fatty acids that are found in all three samples included (C14:0, C16:1, C18:1n9c, C18:1n9, C18:2n6c, C20:4n6, C20:5n3, C22:6n3). The amount of Polyenes (omega-3 and omega-6 fatty acid) is very high in the head region, i.e., 58.82% of total fatty acids, followed by the viscera (45.80%) and the swim bladder (35.57%). The PUFA, docosahexaenoic acid (DHA) was the major fatty acid and found in the head region (20.9%), viscera (17.3%), and swim bladder (14.5%). A significant difference between the DHA content in the head and the swim bladder was found. In the head region, next to DHA, a high percentage of total fatty acids are present, viz., eicosapentaenoic acid (EPA) (13.6%), clupanodinic acid (13.1%), and eicosatrienoic acid (7.2%). The DHA/EPA ratio of the three discards was higher than 1.5. Jalili *et al.* (26) reported that the tiger tooth croaker from Khuzestan coastal waters, Iran, has 11.14 mg/100 g DHA. The fatty acid composition of fish depends not only on species but also on their natural diet, environment, and sex (27). The results indicated that the processing discards can be a good source of omega-3 fatty acids. Among the monounsaturated fatty acids (MUFAs), oleic acid was the predominant fatty acid in all three discards, accounting for almost 6.60–7.26% of total fatty acids. The saturated fatty acid (SFA) percentage found in the samples varied between 0.02% and 10.20%. The major available SFA was palmitic acid. Its content was highest in the swim bladder (30.49%), followed by the viscera (22.21%) and the head (4.74%). A significant difference between concentrations of palmitic acid in the head, viscera, and swim bladder was observed ($p<0.05$). The results obtained in the present study were in agreement with those of the previous studies on two teleost fish organs (28).

Mineral and heavy metal content of tiger tooth croaker discards

The mineral compositions of processing discards from croaker are given in Table 6. Minerals are crucial nutrients, which are needed for normal body functions and processes. According to the Institute of

Medicine the average daily requirement of Na, P, Mn, Zn, Cu, Fe, and Mg are 1,300–1,500, 700, 1.8–2.3, 8–11, 0.9, 8–18 mg/day, and 310–400 g/day, respectively (29). Minerals were found in abundance in the head region compared to the viscera and swim bladder because of the presence of numerous bones. The level of Na in the head was double than that in other two processing discards. Sodium is an important mineral, which is crucially required for bodily functions such as blood-pressure regulation, blood-volume balance, acid–base balance, transmission of nerve impulse, and normal muscle and cell functions. It also reduces the risk of stroke and coronary heart diseases (30). The head portion also contains the highest level of Ca, Mg, and K, i.e., 346.75, 14.89, and 83.57 mg/kg, respectively. The amounts of these elements are significantly different in the head, viscera, and swim bladder of croaker ($p < 0.05$).

Trace elements and heavy metals in fish have gained significant interest from researchers because of their adverse effects on the properties of the raw material and consumers' health. Among the trace elements, some of the elements such as Ni and Pb are used as indicators of environmental pollution, while Fe, Cu, and Cd are accumulated via processing aids and equipment (31). In the head and swim bladder, cadmium was detected below the detection level, and in viscera, only 0.003 mg/kg was detected. Cd causes adverse effects in the human body by damaging the kidneys, bones, and reproductive system. Accumulation of Pb in the body affects the mental ability of children and increases blood pressure, which leads to cardiovascular diseases in adults (32). In croaker waste, the head contains highest Pb concentration (0.23 mg/kg), which is lower than the tolerance levels. Cr is found naturally in plants and animals and it is involved in protein, carbohydrate, and lipid metabolisms; however, excessive amounts of Cr may cause carcinogenic effects (33). Croaker head and viscera contain 0.03 mg/kg of Cr, whereas the swim bladder contains 0.02 mg/kg. No significant difference between Cr content in the head, viscera, and swim bladder was observed ($p > 0.05$). On the basis of the activities of the elements in the biological process of living organisms, they are classified as essential elements. However, these elements are toxic when they exceed their allowable limits. In croaker waste, these elements are at a very lower level than the recommended allowable limit.

The knowledge on the nutritional characteristics of fish processing discards represents a step toward in the process of utilization of fishery waste for value addition and further potential use. The results from this study suggest that the processing discards from tiger tooth croaker are valuable sources of protein, lipid, and minerals. Thus, the discards can serve as secondary raw materials and potential sources of PUFA, bioactive peptides, and minerals, which can be recovered and used for further potential use. Introducing a new processing line for the recovery of high-value bioactive compounds from croaker processing discards may boost the industry to the next level.

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References

1. MPEDA. MPEDA press release export statistics 2014-15 and RGCA. Marine Product Export Development Authority, Cochin, India (2015)
2. CMFRI. CMFRI annual report 2014-2015. Central Marine Fisheries Research Institute, Cochin, India (2015)
3. FAO. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy (2014)
4. Dekkers E, Raghavan S, Kristinsson HG, Marshall MR. Oxidative stability of *Mahi mahi* red muscle dipped in tilapia protein hydrolysates. *Food Chem.* 124: 640–645 (2011)
5. Chakraborty SK, Devadoss P, Manojkumar PP. The fishery biology and stock assessment of jew fish resources of India. pp. 604-616. In: Marine Fisheries Research and Management. Pillai VN, Menon NG (eds). CMFRI, Cochin, India (2000)
6. Siriwardhana N, Kalupahana NS, Moustaid-Moussa N. Health benefits of n-3 polyunsaturated fatty acids: Eicosapentaenoic acid and docosahexaenoic acid. *Adv. Food Nutr. Res.* 65: 211–222 (2012)
7. AOAC. Official methods of analysis of AOAC Intl. 19th ed. Methods 938.08, 940.25, 948.16, and 950.46 Association of Official Analytical Chemists, Gaithersburg, MD, USA (2012)
8. Conway EJ, Byrne A. An absorption apparatus for the micro-determination of certain volatile substances. *Biochem. J.* 27: 419–429 (1933)
9. Shantha NC, Decker EA. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J. AOAC Int.* 77: 421–424 (1994)
10. Raghavan S, Hultin HO. Oxidative stability of a cod-canola oil model system: Effect of order addition of tocopherol and canola oil to washed, minced cod muscle. *J. Aquat. Food Prod. T.* 158: 37–45 (2006)
11. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497–509 (1957)
12. Metcalfe LD, Schmitz AA. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.* 33: 363–364 (1961)
13. Dolan SP, Capar SG. Multi-elements analysis of food by microwave digestion and inductively coupled plasma atomic emission spectroscopy. *J. Food Compos. Anal.* 15: 593–615 (2002)
14. Hultin HO, Kelleher SD. Surimi Proceeding from Dark Muscle Fish. pp. 59–77. In: Surimi and Surimi Seafood. Park JW (ed). Marcel Dekker Inc., New York, NY, USA (2000)
15. Palanikumar M, Ruba Annathai A, Jeya Shakila R, Shanmugam SA. Proximate and major mineral composition of 23 medium sized marine fin fishes landed in the thoothukudi coast of India. *J. Nutr. Food. Sci.* 4: 1–7 (2014)
16. Ersoy B, Hülya S. The proximate composition and fatty acid profiles of edible parts of two freshwater mussels. *Turk. J. Fish. Aquat. Sc.* 10: 71–74 (2010)
17. Nazeer RA, Kumar NSS. Fatty acid composition of horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*). *Asian Pac. J. Trop. Dis.* 2: S933-S936 (2012)
18. Shalini R, Shakila RJ, Jeyasekaran G, Jeevithan E. Sensory, biochemical and bacteriological properties of octopus (*Cistopus indicus*) stored in ice. *J. Food Sci. Technol.* 52: 6763–6769 (2015)
19. Ninan G, Zynudheen AA. Evaluation of quality and shelf life of two commercially important fish species viz., tiger tooth croaker (*Otolithes ruber* Bloch and Schneider) and flathead grey mullet (*Mugil cephalus* Linnaeus) in iced conditions. *P. Natl. A. Sci. India B* 84: 1035–1042 (2014)
20. Ross CF, Smith DM. Use of volatiles as indicators of lipid oxidation in muscle foods. *Compr. Rev. Food Sci. F.* 5: 18–25 (2006)
21. Connell JJ. Intrinsic quality. pp. 5-36. In: Control of Fish Quality. Connell JJ (ed). Fishing News Books Ltd, Oxford, London (1995)
22. Ozogul Y, Ozyurt G, Ozogul F, Kuley E, Polat A. Freshness assessment of European eel (*Anguilla anguilla*) by sensory, chemical and microbiological methods. *Food Chem.* 92: 745–751 (2005)
23. Sharifian S, Zakipour E, Mortazavi MS, Arshadi A. Quality assessment of tiger tooth croaker (*Otolithes ruber*) during ice storage. *Int. J. Food Prop.* 14: 309–318 (2011)
24. Santiago PA. Lipid damage detection during the frozen storage of an underutilized fish species. *Food Res. Int.* 32: 497–502 (1999)
25. Ladikos D, Lougovois V. Lipid oxidation in muscle foods: A review. *Food Chem.* 35: 295–314 (1990)
26. Jalili S, Zoriasatein N, Pour F. Habitat effects on nutritional quality of two marine fish fillets, tiger tooth croaker (*Otolithes ruber*); Four finger threadfins (*Eleutheronema tetradactylum*). *Int. J. Ecosyst.* 4: 119–123 (2014)
27. Celick M, Diler A, Küçükgülmez A. A comparison of the proximate compositions and fatty acid profiles of zander (*Sander lucioperca*) from two different regions and climatic conditions. *Food Chem.* 92: 637–641 (2005)
28. Magalhães BS, Fiamoncini J, Deschamps FC, Curi R, Silva LP. Comparison of

- fatty acid composition in nine organs of the sympatric Antarctic teleost fish species *Nototothenia coriiceps* and *Nototothenia rossii* (Perciformes: Nototheniidae). *Comp. Biochem. Phys. B* 155: 132–137 (2010)
29. Berto A, Da Silva AF, Visentainer JV, Matsushita M, De Souza NE. Proximate compositions, mineral contents and fatty acid compositions of native Amazonian fruits. *Food Res. Int.* 77: 441–449 (2015)
 30. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: Systematic review and meta-analyses. *Brit. Med. J.* 346: 1–20 (2013)
 31. Morgano MA, Rabonato LC, Milani RF, Miyagusku L, Balian SC. Assessment of trace elements in fishes of Japanese foods marketed in São Paulo (Brazil). *Food Control* 22: 778–785 (2011)
 32. Commission of the European Communities, 2001. Commission Regulation (EC) n. 221/2002 of the 6 February 2002 amending regulation (EC) n. 466/2002 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, Brussels, Belgium (2002)
 33. Otten JJ, Hellwig JP, Meyers LD. Dietary reference intakes: The essential guide to nutrient requirements. The National Academy Press. Washington DC, USA (2006)