Thermal Fingerprinting and Nutritional Profiling of Freshwater Fish Muscle: A Comparative Thermogravimetric and Calorimetric Study

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Abstract

The nutritional composition and thermal degradation behavior of freshwater fish muscle are important characteristics for understanding changes in fish tissues during thermal processing. Although the proximate composition of freshwater fish has been extensively studied, comparative information on the thermal stability and degradation behavior of muscle components remains limited. This study characterized the proximate composition and thermal properties of 30 fish representing 10 freshwater species (three biological replicates per species) collected from Chashma Lake and the Indus River, Pakistan. Dorsal muscle samples were separated, oven-dried at 60 °C for 48 h, finely ground, and analyzed for proximate composition and thermal properties using simultaneous thermogravimetric analysis and differential scanning calorimetry (TGA/DSC). Significant interspecific differences were observed for all measured parameters ($p < 0.001$). Moisture, crude protein, crude lipid, and ash contents ranged from 71.80–77.30%, 16.10–20.50%, 2.80–6.80%, and 1.22–3.10%, respectively. *Chitala chitala* and *Hypophthalmichthys nobilis* had comparatively higher protein and lipid contents. The principal stage of thermal degradation occurred between 180 and 400 °C, with species-specific differences in degradation patterns. *Cirrhinus mrigala* exhibited a comparatively slower mass decline and retained a greater proportion of residual char during this stage. The highest enthalpy value was recorded for *H. nobilis* (16,824 J/g), indicating greater energy absorption during thermal degradation under the experimental conditions. *Cirrhinus reba* (26.54%) and *Oreochromis mossambicus* (26.21%) showed the highest residual mass, which was associated with their comparatively higher ash contents. Significant relationships were also observed between proximate composition and thermal characteristics. Overall, the combined application of proximate analysis and TGA/DSC provided a useful approach for differentiating the nutritional composition and thermal degradation characteristics of freshwater fish muscle.

Keywords: Freshwater Fish, Meat, Proximate composition, Thermal stability, TGA/DSC

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Introduction

Fish meat is known globally as one of the most important sources of high quality animal protein to support food and nutritional security globally. Fish meat is much more digestible, has essential amino acids, polyunsaturated fatty acids (especially omega-3 fatty acids), is rich in vitamins and minerals (Khalili Tilami and Sampels, 2018). Eating fish meat on a regular basis has been linked to a variety of health benefits, such as better heart health, cognitive function, and inflammation reduction, as well as other nutritional advantages (Noreen *et al*., 2025, Nousheen *et al*., 2026, Nousheen *et al*., 2025). With a growing world population and growing demand for sustainable protein source, freshwater fisheries have gained more significance in providing low cost and quality nutritious foods, especially in developing countries like Pakistan.Pakistan has large freshwater resources in which a wide variety of indigenous and cultured fishes are found such as fresh water rivers, lakes, reservoirs and barrages (Rafique and Khan, 2012). Of these, the Indus water system and Chashma Lake are important freshwater ecosystems with high ecological and economic values. Chashma lake on Indus river is a Ramsar site which hosts a high number of commercially viable fish species which play significant role in fisheries, employment and food security in the region (Farooq *et al*., 2025a). Comprehensive information about nutritional composition and thermal stability of many freshwater fish species, however, is still limited, especially from the economic point of view. This information is important for assessing fish quality, optimizing processing conditions, fish storage methods, and consumer awareness about the nutritional value of various fish species.Fish muscle nutritional quality is usually evaluated based on the proximate composition of fish, which includes moisture, crude protein, crude lipid and ash content. These are basic parameters that are used to quickly assess the fish quality, freshness, processing characteristics and consumer acceptability (Abraha *et al*., 2018). The moisture content affects the texture, water holding capacity and shelf life, while protein is the key nutritional component, which is important for muscle growth and tissue repair (Sultana *et al*., 2022). The nutritional composition of fish vary in different fish species depending on genetics, feeding habits, habitat, age, reproductive stage, environmental, and seasonal factors (Ahmed *et al*., 2022). Hence, comparative analyses of the proximate composition of fish may be of great importance in the selection of nutritionally superior fish species and in the study of biochemical characteristics of fish species (al, 2011).Conventional proximate analysis data gives quantitative information on the major nutritional components, but does not show the behaviour or stability of these biomolecules when heated (Jamaluddin *et al*., 2026) (Fig. 1).

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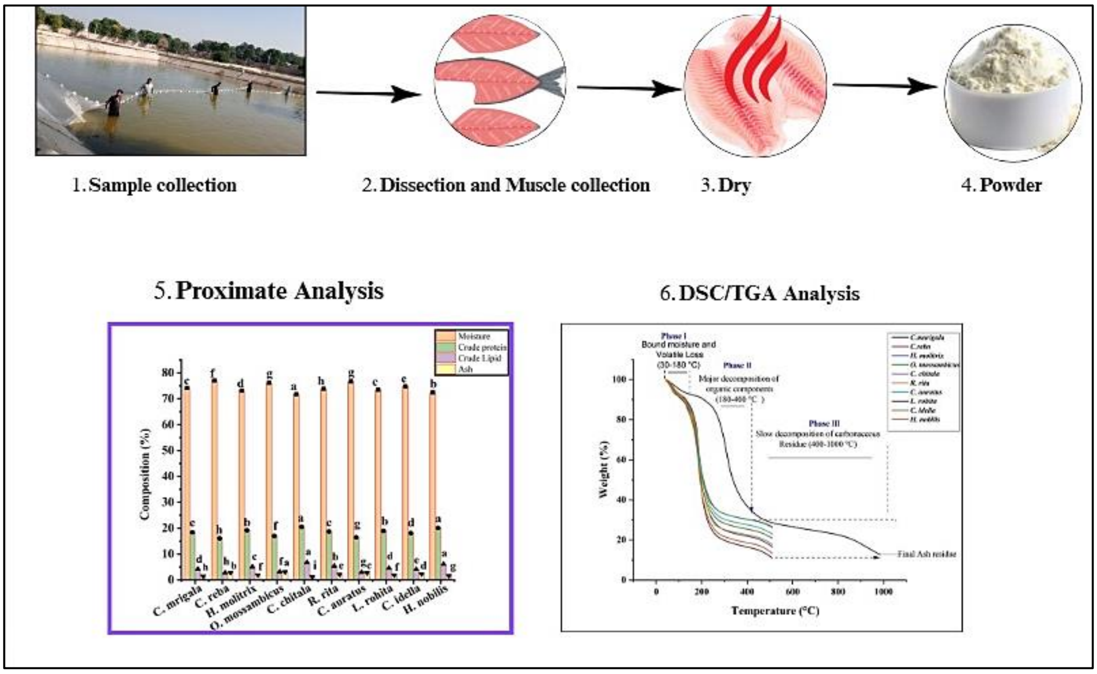

Figure 1: Graphical abstract.

Thermal stability is an important quality attribute due to the extensive physicochemical changes that occur during various processes such as cooking, drying, smoking, roasting, freezing, sterilisations or value addition in industries (Fang *et al*., 2023). Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) have gained attention for studying the thermal stability and degradation behavior of biological materials (Demetzos, 2008). Enthalpy of Denaturation (ΔH) parameter can be use which evaluates the amount of heat energy required to denature certain proteins, in order to assess the thermal stability (ability to withstand structural and chemical disintegration at elevated temperatures) of fish. The greater the ΔH, and the greater the stability of the protein (Durowoju *et al*., 2017). DSC developed by Watson and O'Neill in 1962 and commercially available in 1963, DSC measures heat flow during thermal transition events in a substance, and is used to gain insight into protein denaturation, lipid melting and structural changes during processing and storage (Schick, 2009). In fish muscle studies, DSC is primarily employed for determining the denaturation temperature of proteins constituting vital parameters in meat quality and processing (Schubring, 2009b).On the other hand, TGA measures changes in the sample mass over temperature, providing valuable information on the moisture content, decomposition temperatures, and thermal stability of the biological components such as proteins, fats, and ash (Prime *et al*., 2009). TGA identifies the temperature that the mass of the material is lost and DSC measures the heat absorbed or released during transitions.This combination approach enables a comprehensive understanding of the behaviour of fish muscle during heating, important for the improvement of the fish industry methods of cooking, storing and preservation (Szécsényi and Holló, 2023)..In addition, TGA can determine moisture and ash content of food samples (Pojić *et al*., 2015). Recent studies demonstrate that thermogravimetric analysis (TGA) offers a rapid and low-cost alternative for proximate analysis, producing results comparable to standard methods, with a maximum deviation of only 4.73%, mainly due to moisture variations (Park *et al*., 2022).

The purpose of the present study was to comparatively investigate the proximate composition and thermal degradation behavior of meat from ten economically important freshwater fish species obtained from Chashma Lake, Indus River, Pakistan with respect to these aforementioned knowledge gaps. The moisture, crude protein, crude lipid, and ash content were determined on the basis of conventional proximate analysis, while thermogravimetric analysis (TGA) was used to determine the moisture loss, degradation of organic matter, thermal stability, decomposition pattern and residual mineral content. The present study combines nutritional and thermoanalytical techniques to yield a full picture of the compositional quality and thermal behaviour of the muscle of the fresh water fish. The results will aid the improved understanding of the nutritional value of different species, optimize thermal processing methods, improve the quality management of post-harvest operations, and establish a scientific basis for selecting fish species for human nutrition and industrial food products.

Materials and methods

Study area

The shallow Chashma Barrage wetland (Ramsar designated, 34,089 ha) was built in 1971 on the Indus River close to Mianwali, Pakistan. The depth of the reservoir changes seasonally and the pH is 6.5–7.2. The climate, agriculture and animal husbandry of the surrounding area are dry subtropical. Chashma Lake is an important freshwater ecosystem for fish production of more than 1000 MT/year and water supply for agricultural, animal and human needs. It has a high degree of biodiversity and it is an ecologically and socioeconomically important aquatic habitat (Rind *et al*., 2025).During this study fish was collected from the Indus River—Chashma Lake, Mianwali, Punjab Pakistan.

Collection of fish

Sampling was carried out from 6th July 2025 to 30th August 2025 from various sampling locations of the Chashma Lake (wild). Fish were randomly taken from each sampling site. Ten (n=30) different commercially edible fish species of different trophic levels were selected based on their catch in the fish. The collected fish species (*Cirrhinus mrigala, Cirrhinus reba, Labeo rohita, Rita rita, Ctenopharyngodon idella, Hypophthalmichthys molitrix, Hypophthalmichthys nobilis, Oreochromis mossambicus, Carassius auratus and Chitala chitala*) were

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cleaned by using deionized distilled water and total length and weight were recorded. Then, aseptically packed fish in zip lock labelled bags containing dry ice was sent to fisheries research lab at department of Zoology, University of Mianwali. After this, the fish samples were stored in laboratory at -4°C till further processing.

Sample preparation

The fish muscle tissues were carefully dissected after collecting samples from refrigeration. Dried at constant temperature of 60°C for 48 hours in Drying Oven (Model WHL-25A) the separated muscle tissues were cut into small pieces.The dried muscle tissues were next ground to a fine powder in a hand held mortar and pestle (Fig. 2). An accurate weight of the dried powder was taken for further analysis (Ayanwale *et al*., 2007).

Figure 2: (a)The fish meat is converted into dry muscle (b) and then into fish meat powder.

Proximate analysis

The proximate composition was determined in triplicate for moisture, ash, lipid and protein of the fish meat. Moisture content measurement was done by drying fish sample in oven at 103 °C until a constant weight was reached. Ash content of fish was measured by incinerating the fish at 550 °C .The total lipid was extracted from the muscle tissues using the Bligh and Blighet Dyer method. The fatty matter content was gravimetrically analysed. The Protein content was determined by the micro-Kjeldahl method.(Tenyang *et al*., 2022).

DSC/TGA analysis

Procedure

The thermal stability of fish meat was next analyzed by DSC and TGA, under a nitrogen gas flow of 50 mL/min. and using a sample in alumina crucible as well as an empty alumina crucible as reference (Budianto *et al*., 2025). The temperature was increased from 22.63 °C, which is the room temperature, to 1000 ° C at a constant heating rate of 15 ° C/min under an air atmosphere. The experiment started after weight stabilization of the samples. Use of air cooling at the end of the run until temperature falls below 50°C, default

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calibrations were used. The process was repeated three times with replicates. At all times, a simultaneous thermogravimetric (TGA) and differential scanning calorimetric (DSC) analysis of thermal behavior, thermal degradation and thermal residual mass was performed (Uddin *et al*., 2001).

Statistical analysis

The one-way analysis of variance (ANOVA) was used to compare the mean values among different fish species. If a significant difference was detected ($p<0.05$), a posthoc test called the least significant difference (LSD) test was applied to determine which species were significantly different from the other species.The data were presented graphically for easy comparison using Software (OriginPro 2024).

Results

In the present study, we investigated the nutrient profiling, thermal stability of fish meat from ten different freshwater fish species collected from Chashma Lake of the Indus River, Pakistan.

Traditional proximate composition

The proximate composition analysis of meat from different freshwater fish species showed significant variation ($p<0.001$) in moisture, crude protein, crude lipid, and ash contents among all species (Table 1 and Fig. 3).

Table 1: Traditional proximate composition of freshwater fish meat samples.

Fish Species	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)
C. mrigala	74.20 ± 0.20^{e}	18.40 ± 0.19^{c}	4.20 ± 0.10^{d}	1.45 ± 0.04^{h}
C. reba	77.10 ± 0.20^{a}	16.10 ± 0.20^{h}	2.80 ± 0.11^{h}	2.85 ± 0.05^{b}
H. molitrix	73.20 ± 0.17^{f}	19.20 ± 0.20^{b}	5.10 ± 0.14^{c}	1.92 ± 0.06^{f}
O. mossambicus	76.10 ± 0.18^{c}	17.00 ± 0.13^{f}	3.20 ± 0.14^{f}	3.10 ± 0.09^{a}
C. chitala	71.80 ± 0.20^{h}	20.50 ± 0.11^{a}	6.80 ± 0.19^{a}	1.22 ± 0.04^{i}
R. rita	73.90 ± 0.17^{e}	18.70 ± 0.19^{c}	5.40 ± 0.12^{b}	2.14 ± 0.04^{e}
C. auratus	76.70 ± 0.17^{b}	16.50 ± 0.15^{g}	3.00 ± 0.12^{g}	2.76 ± 0.05^{c}
L. rohita	73.50 ± 0.20^{f}	19.00 ± 0.19^{b}	4.60 ± 0.18^{d}	1.88 ± 0.05^{f}
C. idella	74.80 ± 0.17^{d}	18.10 ± 0.18^{d}	4.10 ± 0.17^{e}	2.30 ± 0.04^{d}
H. nobilis	72.40 ± 0.17^{g}	20.10 ± 0.17^{a}	6.10 ± 0.10^{a}	1.60 ± 0.04^{g}
F value	44.0	67.3	40.6	39.6
p value	0.000***	0.000***	0.000***	0.000***

Data are reported as mean ± SD (n=3).
*$p<0.05$; **$p<0.01$; ***$p<0.001$ and different superscript letters within a column indicate statistically significant differences ($p<0.05$).

The moisture content of the analyzed freshwater fish species ranged from 71.80% to 77.10%, showing significant interspecific variation. The highest moisture content was recorded in *C. reba* (77.10%), followed by *C. auratus* (76.70%) and *O. mossambicus* (76.10%). In contrast, the lowest moisture content was observed in *C. chitala* (71.80%), followed by *H. nobilis* (72.40%) and *H. molitrix* (73.20%).Crude protein content varied between 16.10±0.17% and 20.50±0.17%.

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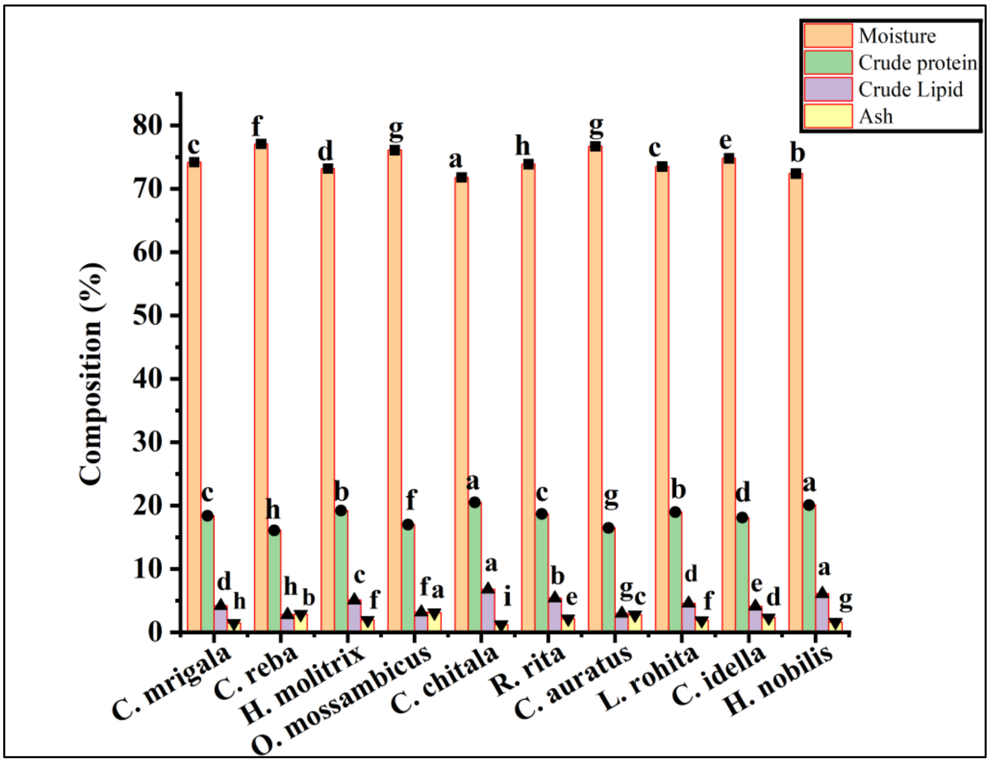

Figure 3: Comparative proximate composition of freshwater fish muscle tissues.

The highest protein values were recorded in *C. chitala* and *H. nobilis*, indicating their superior nutritional quality and importance as rich protein sources for human consumption.*C. reba* and *C. auratus*, on the other hand, had relatively low protein content..Crude lipid content ranged from 2.80 ± 0.10% in *C. reba* to 6.80 ± 0.10% in *C. chitala*. The higher lipid content of *C. chitala* and *H. nobilis* indicates high energy storage and better caloric content than the lower lipid content of *C. reba* and *C. auratus* which makes them relatively lean fish species. The concentration of lipids is an important quality parameter as it affects flavor, texture and nutritional value of the product, including essential fatty acids.

The total mineral content (ash content) varied significantly between species and ranged from 1.22 ± 0.04% in *C. chitala* to 3.10 ± 0.09% in *O. mossambicus*. The presence of higher ash values in *O. mossambicus* and *C. reba* suggest a relatively higher mineral deposition in the muscle tissues. The high values of the F-ratios of the various fish species for moisture (40.0), crude protein (67.3), crude lipid (40.6) and ash (39.6) further support the highly significant biochemical variability among the fish species studied. In conclusion, the findings indicate that all the freshwater fish species studied have great nutritional potential but *C. chitala* and *H. nobilis* have comparatively good protein and lipid profile and hence they can be considered as nutritionally valuable species.

DSC/TGA 1000 °C

The DSC-TGA (Fig. 4a-j) analysis gave an in-depth insight into the thermal

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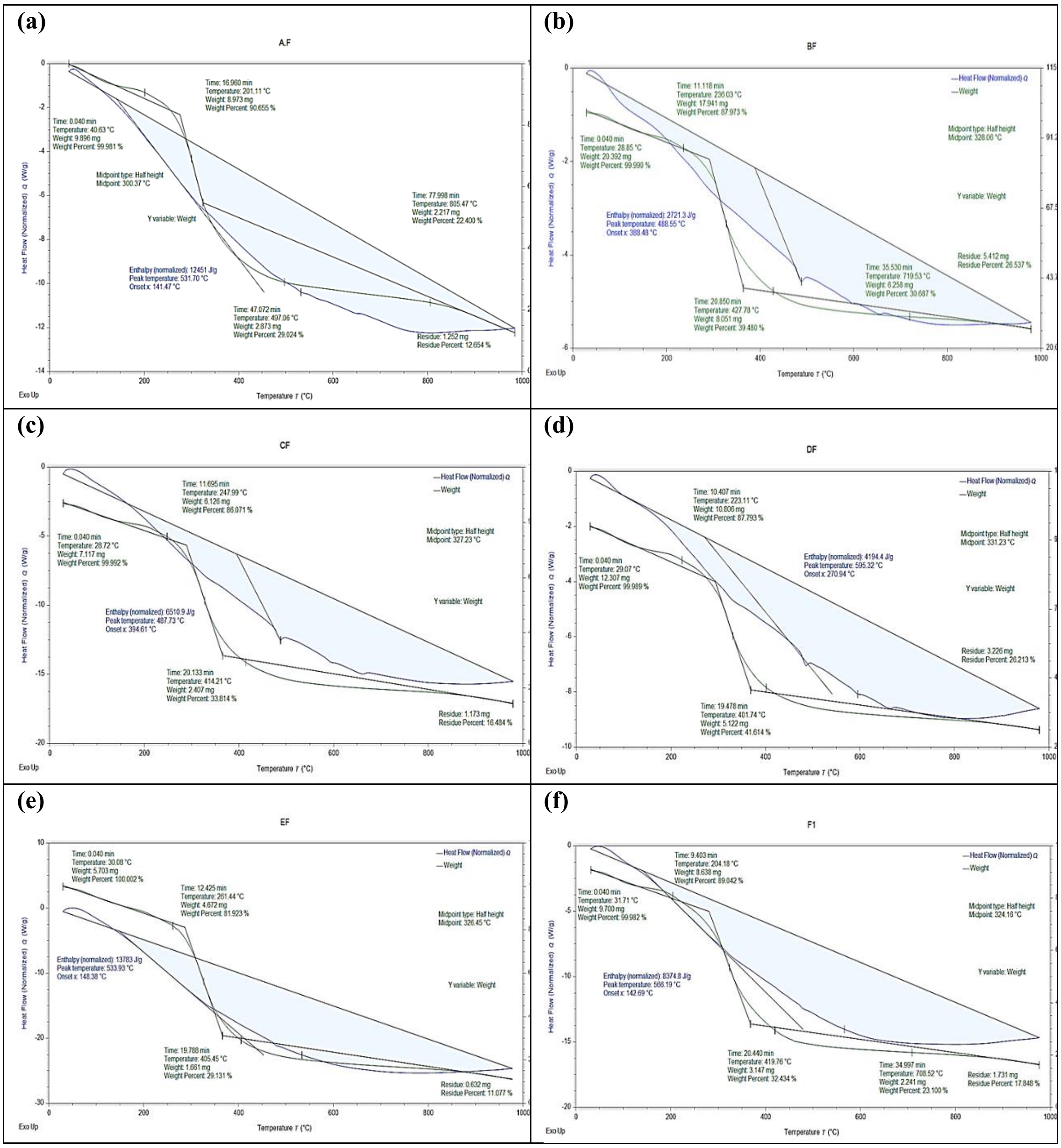
analyze the behavior and stability of muscle tissues of the studied freshwater fish species by assessing them various parameters, such as residue (ash %), enthalpy, temperature at onset, maximum degradation temperature, glass transition temperature (Tg) (Table 2).



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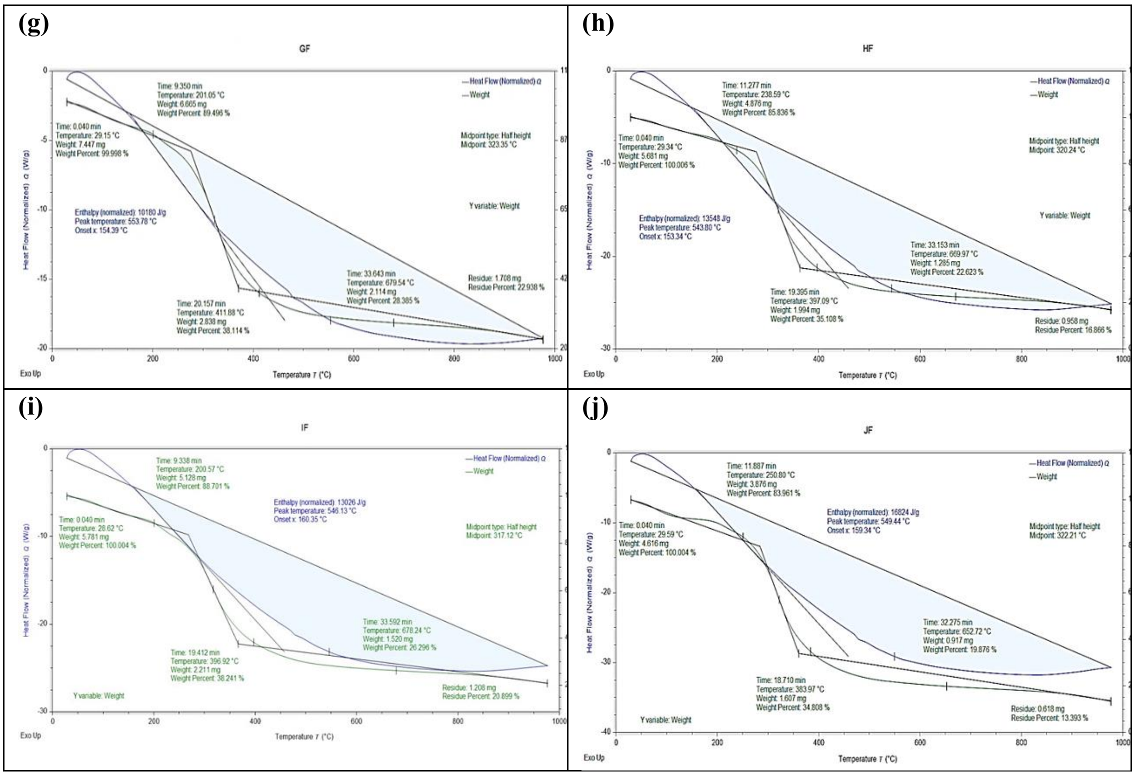

Figure 4: Comparative DSC–TGA thermograms illustrating the thermal degradation behavior and thermal stability of muscle tissues from ten freshwater fish species: (a) *C. mrigala*, (b) *C.reba*, (c) *H.molitrix*, (d) *O. mossambicus*, (e) *C.chitala*, (f) *R.rita*, (g) *C.auratus*, (h) *L.rohita*, (i) *C.idella*, and (j) *H.nobilis*.

Table 2: TGA/DSC key thermal parameters detected in the present study at 1000°C.

Fish Species	Residue (Ash %)	Enthalpy (J/g)	Onset Temp (°C)	Peak Temp (°C)	Tg (°C)
C. mrigala	12.65 ± 0.05^{d}	12451 ± 0.20^{b}	141.47 ± 0.12^{f}	531.70 ± 0.10^{c}	300.37 ± 0.10^{f}
C. reba	26.54 ± 0.06^{a}	2721 ± 0.10^{f}	388.48 ± 0.11^{b}	488.55 ± 0.13^{f}	328.06 ± 0.11^{b}
H. molitrix	16.48 ± 0.07^{c}	6510 ± 0.20^{e}	394.61 ± 0.14^{a}	487.73 ± 0.15^{f}	327.23 ± 0.17^{c}
O. mossambicus	26.21 ± 0.02^{a}	4194 ± 0.10^{f}	270.94 ± 0.15^{c}	595.32 ± 0.15^{a}	331.23 ± 0.11^{a}
C. chitala	11.08 ± 0.03^{d}	13783 ± 0.20^{b}	148.38 ± 0.18^{e}	533.93 ± 0.11^{c}	326.45 ± 0.11^{d}
R. rita	17.85 ± 0.04^{c}	8374 ± 0.20^{d}	142.69 ± 0.11^{f}	566.19 ± 0.11^{b}	324.16 ± 0.13^{e}
C. auratus	22.94 ± 0.08^{b}	10180 ± 0.22^{c}	154.39 ± 0.16^{d}	553.78 ± 0.16^{b}	323.35 ± 0.12^{e}
L. rohita	16.87 ± 0.07^{c}	13548 ± 0.20^{b}	153.34 ± 0.12^{d}	543.80 ± 0.13^{c}	320.24 ± 0.16^{g}
C. idella	20.90 ± 0.04^{bc}	13026 ± 0.23^{b}	160.35 ± 0.19^{d}	546.13 ± 0.12^{c}	317.12 ± 0.18^{h}
H. nobilis	13.39 ± 0.01^{d}	16824 ± 0.25^{a}	159.34 ± 0.11^{d}	549.44 ± 0.17^{b}	322.21 ± 0.12^{f}
F value	24.86	68.72	81.35	56.91	39.48
P value	**0.000*****	**0.000*****	0.000***	0.000***	**0.000*****

Data are reported as mean ± SD (n=3).
**p*<0.05; ***p*<0.01; ****p*<0.001 and different superscript letters within a column indicate statistically significant differences (*p*<0.05).

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The residue (ash %), enthalpy, onset temperature, peak temperature and glass transition temperature (Tg) of the ten freshwater fish species exhibited significant difference ($p<0.001$). There was a significant variation in the ash content of the different materials ranging from 11.08±0.03% in *C. chitala* to 26.54±0.06% in *C. reba*, reflecting the level of inorganic content after thermal degradation. Similarly, enthalpy values varied markedly among species, with the highest value observed in *H. nobilis* (16824±0.25 J/g), followed by *C. chitala* (13783±0.20 J/g) and *L. rohita* (13548±0.20 J/g), whereas *C. reba* exhibited the lowest enthalpy (2721±0.10 J/g). The results show that the higher the enthalpy value, the more heat was needed to denature the protein species, which means that the muscle proteins were more thermally stable.The temperature at which thermal degradation began (onset temperature) also varied greatly between the species. The highest onset temperature was found in the case of *H. molitrix* (394.61±0.14°C), followed by *C. reba* (388.48±0.11°C) which indicated that the degradation process occurs later, and the compounds are more resistant to thermal decomposition. However, the onset temperature for *C. mrigala* (141.47±0.12°C) and *R. rita* (142.69±0.11°C) were the lowest showing that thermal degradation started at comparatively low temperatures. The peak temperature values were found to be between 487.73±0.15°C in *H. molitrix* and 595.32±0.15°C in *O. mossambicus*, showing significant differences in the maximum decomposition temperature of the fish species studied.The glass transition temperature (Tg) ranged from 300.37±0.10°C in *C. mrigala* to 331.23±0.11°C in *O. mossambicus*. The higher the Tg value, the more rigid the muscle matrix and the better the resistance to heat; the lower the Tg value, the more the molecules move around when heated, and the less heat resistance the muscle matrix has. In general the results show high inter-specific variability in thermal behavior and confirm that each of the fish species has a different thermal degradation profile. These differences probably relate to the differences in the muscle protein structure, the presence of lipids, moisture and mineral composition. All the thermal parameters measured were also significantly different between the fish species ($F=24.86–81.35$; $p<0.001$) according to different superscript letters within each column in One-way ANOVA.

TGA nutrient degradation behavior

Thermal degradation patterns of meat from ten freshwater fish species were different in three stages of thermal degradation, as shown in the thermogravimetric analysis (TGA) (Table 3 and Fig. 5). In the Phase I which corresponds to the loss of free and bound moisture and volatile compounds, the weight loss in the tested organisms varied from 9.33±0.11% for *C. mrigala* to 18.08±0.15% for *C. chitala*. The initial weight loss of the three fishes *C.*

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chitala, H. nobilis (16.04±0.13%) and *L. rohita* (14.17±0.12%) suggests that higher moisture content and volatile content in these fishes whereas the lowest weight loss in *C. mrigala* shows lower moisture content and less volatiles during heating. The second degradation phase (150 to 400°C) was the most significant thermal decomposition of proteins, lipids and other organic components. This stage accounted for the highest mass loss in all species, ranging from 57.28±0.14% in *C. reba* to 70.84±0.22% in *C. chitala. H. molitrix* (69.59±0.21%), *C. mrigala* (68.25±0.17%) and *R. rita* (65.94±0.22%) showed high degradation of organic matter, which showed a higher percentage of thermally degradable biomolecules.

Table 3: Thermal decomposition–based component distribution of fish meat samples.

Fish Species	Phase I	Phase II	Phase III	Phase IV
	Moisture & Volatile Loss (%)	Organic Degradation (%)	Char Degradation (%)	Formation of Ash (%)
	(30–150°C)	(150–400°C)	(400–650°C)	(650–1000°C)
C. mrigala	9.33 ± 0.11^{d}	68.25 ± 0.17^{a}	9.77 ± 0.14^{a}	12.65 ± 0.03^{d}
C. reba	12.02 ± 0.10^{c}	57.28 ± 0.14^{e}	4.16 ± 0.12^{d}	26.54 ± 0.03^{a}
H. molitrix	13.92 ± 0.14^{c}	69.59 ± 0.21^{a}	0.01 ± 0.15^{e}	16.48 ± 0.05^{c}
O. mossambicus	12.20 ± 0.11^{c}	61.58 ± 0.17^{d}	0.01 ± 0.12^{e}	26.21 ± 0.04^{a}
C. chitala	18.08 ± 0.15^{a}	70.84 ± 0.22^{a}	0.00 ± 0.11^{d}	11.08 ± 0.03^{d}
R. rita	10.94 ± 0.12^{d}	65.94 ± 0.22^{b}	5.27 ± 0.14^{bc}	17.85 ± 0.05^{c}
C. auratus	10.50 ± 0.10^{d}	61.11 ± 0.21^{d}	5.45 ± 0.13^{bc}	22.94 ± 0.04^{b}
L. rohita	14.17 ± 0.12^{b}	63.22 ± 0.18^{bc}	5.74 ± 0.14^{b}	16.87 ± 0.05^{c}
C. idella	11.30 ± 0.11^{d}	62.41 ± 0.16^{d}	5.39 ± 0.12^{b}	20.90 ± 0.04^{bc}
H. nobilis	16.04 ± 0.13^{ab}	64.08 ± 0.18^{bc}	6.49 ± 0.13^{b}	13.39 ± 0.05^{d}
F-value	42.68	58.91	73.45	24.86
P-value	0.000***	0.000***	0.000***	0.000***

Data are reported as mean ± SD (n=3).
*$p<0.05$; **$p<0.01$; ***$p<0.001$ and different superscript letters within a column indicate statistically significant differences ($p<0.05$).

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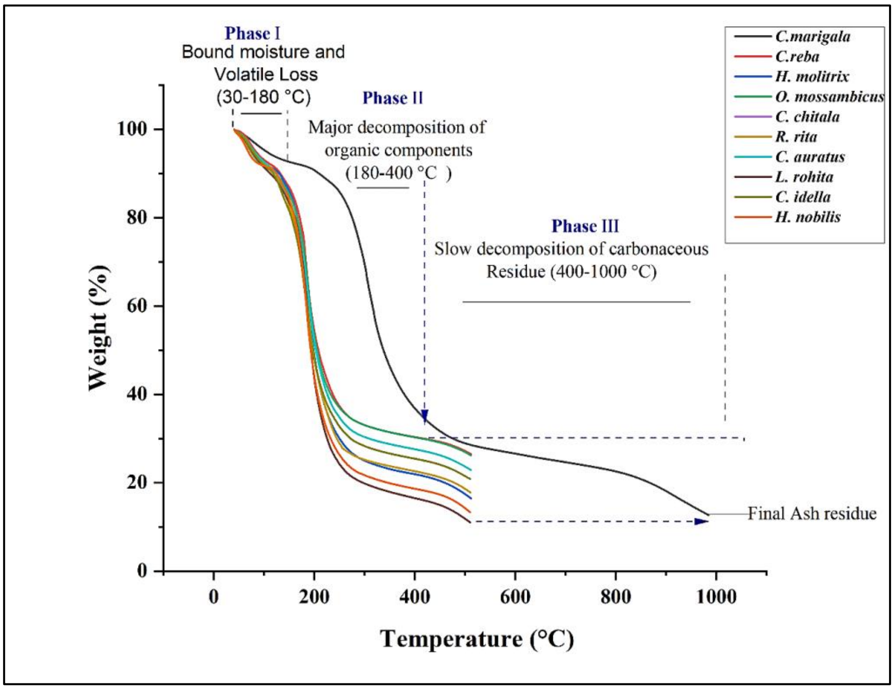

Figure 5: Visual representation of thermal Degradation Phases of fish nutrients.

By contrast, the comparatively small weight lost for *C. reba*, *O. mossambicus* and *C. auratus* indicates relatively low organic content or high thermal resistance of their muscle components. Weight loss was variable among species during Phase III (400 - 650°C) where the carbonization and breakdown of the remaining organic matrix occurred. *C. mrigala* showed the highest weight loss (9.77±0.14%), followed by *H. nobilis* (6.49±0.13%) and *L. rohita* (5.74±0.14%), indicating continued degradation of carbonaceous residues. The degradation in weight loss was very low with negligible loss in weight (0.00–0.01%) for *C. chitala* and *O. mossambicus* in the second phase indicating that most of the organic matter available for degradation had already been broken down. The final residual ash content (>650°C), representing the thermally stable inorganic mineral fraction, ranged from 11.08 ± 0.03% in *C. chitala* to 26.54±0.03% in *C. reba*. Comparatively higher value of ash in *C. reba* (26.54%), *O. mossambicus* (26.21%) and *C. auratus* (22.94%) showed more mineral constituents content of the fish samples while comparatively lower value of ash in *C. chitala* (11.08%), *C. mrigala* (12.65%) and *H. nobilis* (13.39%) showed comparatively lower inorganic content of the fish samples. The slower thermal degradation of *C.mrigala* may be attributed to the formation of a relatively more stable char layer during heating, which acted as a protective barrier and delayed further decomposition of the muscle matrix.All the thermal Phases measured were also significantly different between the fish species (F=24.86–73.45; $p<0.001$) according to different superscript letters within each column in One-way ANOVA.

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In general, the results showed that the thermal degradation behavior varies significantly among the studied species of freshwater fish, due to differences in the moisture content, organic content, carbonization properties, and mineral residue of these fish species.

Correlation heatmap among thermal and compositional parameters

A significant correlation was found between the proximate composition and thermal characteristics of the meat from species of fresh water fish by using the Pearson correlation analysis (Fig. 6). As for the moisture content, it had a perfect negative correlation with crude protein (r= −1.00) and a very strong negative correlation with crude lipid (r = −0.96), suggesting a negative relationship between moisture content and crude protein or lipid contents of different fish species. The moisture, on the other hand, presented strong positive correlation with ash (0.90) and residue (0.91) and moderate negative correlation with enthalpy (−0.67). The crude protein was significantly positively correlated with the crude lipid (r= 0.97) and enthalpy (r= 0.69), while the crude protein was significantly negatively correlated with ash (r= −0.89) and residue (r= −0.90). Crude lipid also had a positive correlation with enthalpy (r = 0.62) and negative correlations with ash (r= −0.84) and residue (r= −0.86).The ash content showed almost perfect positive correlation with residue (r = 0.99), which indicates that the mineral content is the main factor associated with the residual mass after thermal degradation. The enthalpy showed negative correlation with ash (r = −0.74), residue (r = −0.75) and onset temperature (r= −0.78), which means that the greater the mineral content, the lower is the amount of energy required during the thermal transitions. Onset temperature had a moderate positive correlation with Tg (r= 0.49) and a moderate negative correlation with the peak temperature (r = −0.60). The maximum degradation temperature (peak temperature) was relatively independent of nutritional composition, as indicated by weak correlations with the majority of proximate composition parameters (r≤0.23).

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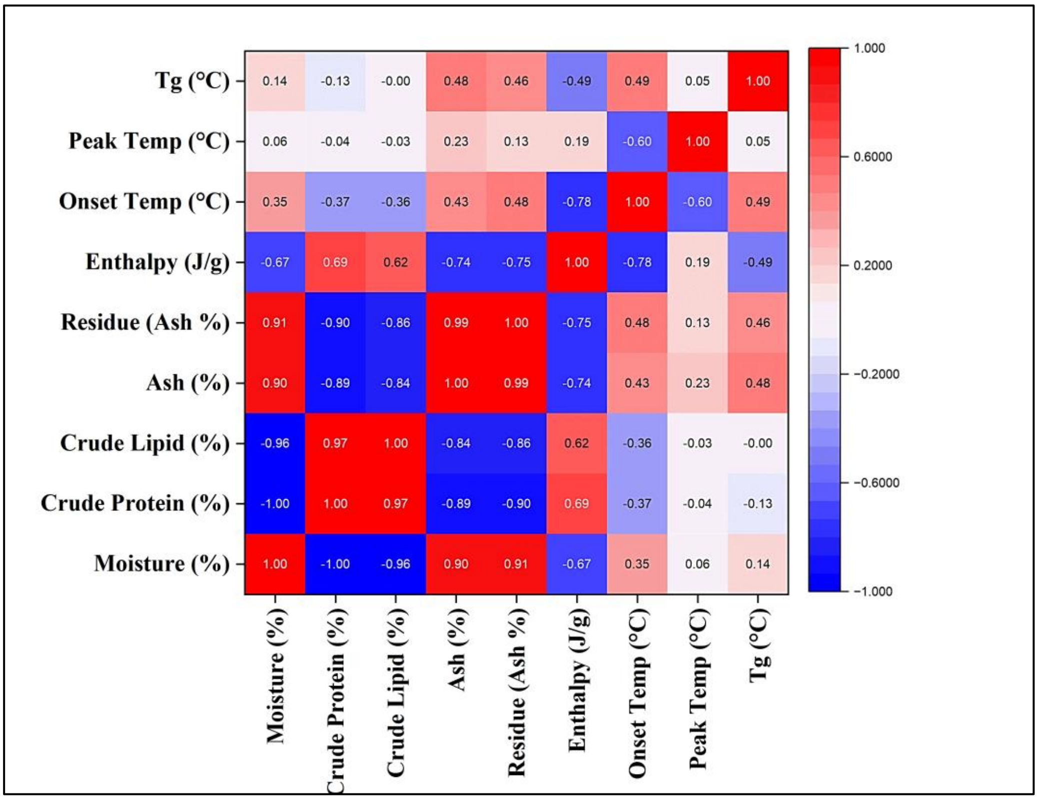

Figure 6**: Pearson correlation heatmap illustrating the associations among proximate composition and thermal degradation parameters.**

Discussion

The present study combined proximate composition and thermal characterization (DSC and TGA) to fully assess the functional and processing stability of fish meat. The proximate analysis determined the primary macronutrients (moisture, protein, lipid and ash) that directly informed the thermal behaviour of the meat matrix. In conjunction, Thermogravimetric Analysis (TGA) showed multiple stages of thermal degradation, which was well correlated with the loss of moisture, protein pyrolysis and remaining inorganic ash observed during the proximate analysis. These combined findings fill the gap between chemical composition and structural stability and give important benchmark data to optimize the industrial processing, drying and thermal preservation methods for fish muscle.In case of DSC/TGA the research are limited in a present time but we study thermal stability of fish meat extensively and find that each fish thermogram is different from other comparable fish.In the current study, the thermal behavior of fish muscle of ten freshwater species was analyzed. The results indicated that the initial weight, the pattern of thermal degradation, the percentage of residue, and enthalpy of protein denaturation were different for different species. Moisture, organic matter and ash content of the species differed significantly (9.33–18.08%, 57.28–70.84% and 11.08–26.54%, respectively), which shows a difference in biochemical

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composition and structural organization of muscle macromolecules. As a result, species like *C.chitala, H. molitrix and H. nobilis* had higher organic matter and lower ash content which in turn implied that there was more thermally labile biomolecules such as proteins and lipids. *C.reba* and *O.mossambicus*, on the other hand, had higher ash contents and lower organic fractions, indicating a higher mineral content and a relatively low organic stability.The results of these tests are in agreement with previous thermal tests performed on biological materials, where findings suggest that biological materials with high protein and lipid content have a high mass loss in the medium temperature range (200–500°C) while mineral rich samples have high residue at high temperatures (Carrier *et al*., 2011).The high negative correlation between the ash content and organic matter content (r= –0.85) obtained in this work is consistent with (Guo *et al*., 2016), who reported that thermally recalcitrant fractions and mineral components dominate the high-temperature region, while labile organic matter governs early and intermediate mass loss. Likewise, the mild negative correlation with moisture indicates that tissues with higher mineral contents hold less moisture and will have less loss of mass due to dehydration.DSC profiles also showed species specific differences in enthalpy, which reflects the differences in thermal stability and molecular organization. The species with higher enthalpy values (*H. nobilis*, *C. chitala* and *L. rohita*) indicated higher tendency of intermolecular interactions and resistance against thermal denaturation of muscle proteins. The behavior is similar to that observed by (Durowoju *et al*., 2017), who demonstrated that higher enthalpy reflects enhanced structural integrity and stability of protein matrices.

In contrast, *O. mossambicus*, and *C. reba*, which have lower enthalpy and higher ash content, had lower thermal resilience, suggesting less protein network strength and higher thermal degradation.In another study, dried skipjack tuna (*Katsuwonus pelamis*), Katsuobushi, is a traditional Japanese dried fish product that is boiled and smoke-dried, and is reported to be a glassy material because of its low moisture content. They noted that its glass transition temperature (Tg), which depends on the moisture content, varied from 11°C to 165°C (Hashimoto *et al*., 2003). The results of this moisture-dependent Tg behavior are comparable to our results, as low-moisture fish muscles have higher Tg values due to the dry state of fish muscles during analysis.When moisture is low molecules are highly packed and more energy is need to initiate to reach a mobility and as a result high Tg. The removal of water from the fish muscle also led to the stabilization of the nutrients, including the amino acids and fatty acids, which were more resistant to degradation (Oveland *et al*., 2024).

Three major endothermic events in fish and shellfish meat that were attributed to myosin, sarcoplasmic proteins or act myosin complex and actin. Likewise, in our study, specific

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thermal transitions were also observed in the DSC thermograms of the fish muscle, with different onset and peak temperatures of various fishes (*C. mrigala, C. reba* and *H. molitrix*) that could be attributed to differences in protein composition and thermal stability. The thermal sensitivities of fish proteins, particularly those of actin and myosin mentioned in both studies, are confirmed, supporting the findings of the previous section (Uddin *et al*., 2001).

Thermal denaturation of shrimp muscle by DSC and observed that the enthalpy of the protein denaturation generally decreased significantly with increasing heating temperature (30-80°C). This means that the proteins are unfolding and losing their structure over time (Schubring, 2009a). The same was seen in our study, where the enthalpy values of the freshwater fishes recorded different endothermic peaks. A decrease in enthalpy in some samples (e.g., *C. reba* and *H. molitrix*) might be due to partial denaturation or loss in protein stability in the presence of heat, similar to what was observed in shrimp. The changes in the DSC curve between various stages of the thermal process are well correlated with the changes in meat appearance and color of the fish and shellfish, making DSC a valuable tool for evaluating the quality of fish and shellfish during thermal process.

DSC and TGA were used to test the oxidative stability of the hoki and tuna oils and noted the presence of three stages of thermal degradation which corresponded to the oxidation of the PUFA, MUFA and SFA fractions (Tengku-Rozaina and Birch, 2016). In the same manner, fish muscle DSC and TGA thermograms revealed multiple thermal transitions and degradation stages which reflect progressive degradation of protein and lipid components. Defined onset and peak temperatures were detected in our fish species, which is consistent with the thermal profile of fish oils and suggests the sensitivity of aquatic tissues to oxidation and thermal stress. The comparisons highlight the value of thermal analysis for lipid-rich materials such as fish oil and protein-rich materials such as muscle, for quality assessment, shelf life prediction and thermal stability profiling. The more ordered the secondary structures, the stronger the intramolecular interactions, the higher the ΔH.

Enthalpy values measured by DSC are a direct measure of the energy necessary to break the molecular interactions between the feed and muscle matrix, specifically protein–lipid interactions. The higher the enthalpy the more complex and energy-rich the structure: a larger amount of thermal energy will be needed to denature it (higher thermal stability). The enthalpy value of the diets containing higher lipid inclusion ratios (especially L9 and L12) was much higher (up to 13,297 J/g) than that of the low-lipid diets, which indicated higher intermolecular bonding and more stable biochemical matrix of the high-lipid diets. This increase in enthalpy was accompanied by higher peak and onset temperatures, thus supporting the argument that lipid enrichment

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contributes to the resistance of muscle tissue to thermal degradation (Farooq *et al*., 2025b). These results are very similar to our results, as samples with a higher enthalpy also had a higher degree of thermal resistance and a longer decomposition delay, meaning that the molecular stability depends on the dietary composition. Therefore, the enthalpy is a good thermal indicator that correlates with feed formulation and structural integrity, and energy dynamics of fish muscle and corroborates the mechanistic trends observed in the present study.

Biofield energy treatment brought about significant changes in fish peptone. The treated sample had a slight increase in particle size and a decrease in surface area, which indicates that there was rearrangement of the molecules. Thermal stability increased, the treated sample exhibited higher transition enthalpy (–71.14 J/g to –105.32 J/g) and higher degradation temperature (213.31°C to 221.38°C) than the untreated sample, and had less weight loss. Based on the above findings, it can be concluded that biofield energy treatment could improve the thermal stability of fish peptone for more suitable use in industries (Trivedi *et al*., 2015). This study also revealed that when enthalpy value higher it is indication of thermally stable molecules. The TGA results provide information on mass loss profiles and mass differences between the species in addition to the DSC results. Typically, the loss of mass is presented on a curve with a drop in mass due to moisture evaporation, then decomposition of organic matter (lipids, proteins) and finally inorganic residue. Species with high thermal stability (high ΔH) show delayed onset of decomposition and high residual ash content, which are characteristics of more resistant organic fractions. Early decomposition (oxidation, chain scission) can result in faster mass loss in species with higher levels of lipid unsaturation or labile linkages.

(TGA) and (DSC) of high impact polystyrene (HIPS), pineapple leaf fibre (PALF) and the composites, and concluded that the thermal stability was enhanced as a result of the addition of natural fibres. Neat HIPS degraded only in a single stage between 341-483 °C with only 2.4% of the mass remaining and PALF and HIPS/PALF composites showed multi-stage degradation trend and higher residual mass because of presence of a cellulose-lignin network in the fibres which delayed the thermal decomposition. TGA was conducted from 25°C to 800°C at 20°C/min under a flow of nitrogen (50 mL/min) in order to monitor the weight loss of the samples, whereas DSC was performed from 25°C to 300°C to determine the thermal transition and enthalpy changes (Siregar *et al*., 2011). These findings are consistent with those from our muscle study, which revealed that samples with higher enthalpy and residual mass, and less lipid interference, had a denser protein–lipid matrix and possessed greater thermal stability. In both cases, it has been found that the intrinsic structural composition (fibre content in a composite, protein–lipid density in

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muscle) directly controls thermal resistance and stability.The thermal behavior found in this study is consistent with the STDN-based study conducted by (Guo *et al*., 2016), that is a slow thermal degradation under nitrogen atmosphere. The thermo-chemical analysis indicated that mass loss and energy absorbed were higher for the species with higher protein and lipid content at lower temperatures, suggesting the decomposition of labile organic components; while mass loss and energy absorbed were lower for species with higher ash content, suggesting the dominance of minerals. The interspecific variation in thermal peaks reinforces the notion of a species-specific thermal "fingerprint" and shows that controlled thermal degradation can be used to reliably distinguish various biological materials, depending on the quality and stability of organic matter.The quality of fish meat protein and the beneficial lipids make fish muscle a valuable product. Nevertheless, proteins can be affected by cooking, sterilizing, drying, freezing, etc. and become less digestible or change in texture. Functional integrity may be preserved during processing and storage for species with proteins that are more thermally stable. Some species may be more vulnerable to denaturation, aggregation or nutritional losses during heating. Furthermore, variation in lipid content and stability may affect how the product is affected by oxidation during storage. Unsaturated lipids are easily oxidized to form reactive species which can cause damage to the protein structure. Also species with higher and more stable protein may indicate better intrinsic antioxidant protection or antioxidant properties of the lipid composition against peroxidation.

The proximate composition results in this study are very similar to those of the Indus river freshwater fish species. In that case, 8 species of fish from the Indus river were studied and it was observed that moisture content varied from 59.95% to 79.45%, ash content varied from 0.05% to 4.95%, crude protein was found to be from 17% to 20.09% and lipid content was found to be 0.85% to 18.32% which were statistically significant ($P < 0.05$) among different species of fish (Memon *et al*., 2010). The present results fall well within these ranges, with moisture from 71.80% to 77.30% ,protein from 16.10% to 20.50%, and ash from 1.22% to 3.10%. This agreement, particularly in protein content, substantiates the pattern of species examined here which is typical of the compositional pattern of fish of Indus River. The large interspecies variation ($p<0.001$) also supports that species identity and not shared habitat is the primary determinant of the proximate composition of freshwater fish.

Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) are powerful thermal analytical techniques that should be used across aquaculture and various industrial sectors to evaluate the thermal behavior, stability, and compositional properties of biological and chemical materials. Overall, DSC and TGA provide

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accurate, reliable, and detailed thermal fingerprints that enhance quality assurance, material characterization, feed optimization, and product development across aquaculture and industrial systems.

Conclusion

The present study aims to make a detailed analysis of nutritional composition and thermal degradation properties of meat of 10 species of freshwater fishes sampled from Chashma Lake, Indus River, Pakistan. The biochemical composition of fish muscle was found to have significant interspecific differences in respect of proximate composition and thermal characteristics, which implies that the thermal stability and degradation behavior are strongly affected by the biochemical composition. The nutritional quality and high energy requirement for thermal degradation of protein and lipid contents of the species were higher which indicated that they have better thermal stability, in the case of greater protein and lipid contents, like *C.chitala* and *H.nobilis*. In contrast, species with higher mineral content (such as *C.reba* and *O.mossambicus*) exhibited higher residual ash after thermally treating, indicating a higher thermally stable inorganic content.The thermogravimetric and differential scanning calorimetric analysis were able to detect the different stages of moisture evaporation, organic matter decomposition and carbonization, giving detailed information on the thermal behavior of freshwater fish meat. The correlations between proximate compositions and thermal parameters are good, which indicated the high correlations between moisture, protein, lipid and mineral compositions and the degradation profile and residual mass of fish tissues.In overall, the proximate analysis along with TGA/DSC is an effective and useful method for evaluation of nutritional value and thermal stability of freshwater fish meat. The results enable greater knowledge about biochemical and thermal properties of each species and are a valuable scientific information for fish meat quality evaluation, optimization of fish processing, development of new fish products and further research on thermal properties and nutritional value of aquatic food resources.

Author contribution

Saima Nousheen: Conceptualization of the study, Data curation, Formal analysis, Methodology development, Writing original draft, Visualization of results. Imrana Amir: Overall supervision, Conceptualization, Methodology design, Validation, Project administration, Resource acquisition, Writing review & editing, Critical intellectual revision of the manuscript. Hira Bukhat: Data curation, Formal analysis.Shamsa Rubab:Critical Revision and editing of manuscript.Umar Farooq and Zeeshanullah : Field investigation, Data collection, Preliminary data processing.

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Ethical approval

This study followed ARRIVE guidelines for reporting animal research.

Conflict of interest

The authors declare that they have no competing interests.

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Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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