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# Nutritional Profile, Antioxidant Capacity and Physicochemical Properties of Processed *Labeo bata*

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## Abstract

Fish has been a potential food source for humans. *Labeo bata* is a commonly consumed fresh water fish species, particularly in South India. This study explored the effect of different cooking (boiling, steaming, microwaving, and frying) on structural, morphology, nutritional profile, and *in-vitro* antioxidant activity of *Labeo bata*. All these processing methods reduced the water content and improved the quantity of major nutrients. Foremost changes were obtained in the FT-IR spectra of processed fillets with respect to the intensity and shifting of major bands in specific regions. Amino acids, fatty acid profile and mineral content values were also varied significantly ($P$<0.05) upon processing. Physico-chemical characteristics including cooking loss, water activity, color, and pH of both raw and cooked Indian mackerel differed significantly ($P$<0.05). *In-vitro* antioxidant activity was analyzed using the QUENCHER procedure. The DPPH and ABTS scavenging capacity of cooked fillets ranged from 14.8 to 28.0 and from 19.3 to 34.5 mmol Trolox Eq./kg.fish dry basis, respectively.

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## Introduction

Across culture, time, and space, fish has been a potential food source for humans. It has been documented as an exceptional source of protein, with high nutritional value. Among the available inland fish varieties, fresh water fish are available in abundance and are consumed more across peninsular India. *Labeo bata* is a frequently consumed fresh water fish species, particularly in South India, and it is extensively distributed in the tropical Indo-Pacific region (Jayasankar et al. 2004). This species yields significant revenue in South India, with an estimated annual production of 1,061 metric tons reported in 2015-2016 in Puducherry (Sivadas et al. 2016). This fish species has a lengthy body with a slightly thick appearance. Consumers favor it because it has been well identified for its taste, easy cooking ability, high nutrient content in muscle tissue, and low cost owing to its abundant availability (Mohan et al. 2008; Sahena et al., 2010). This study aimed to make consumers more aware of the

nutritive potential of *Labeo bata*. This study also investigated the optimal cooking method that preserves the nutrient content of the fish because cooking methods and temperature considerably affect the nutrient content and antioxidant capacity of fish. Cooking methods involving heat, including boiling, steaming, frying, and microwaving, can alter the structure of food components. Hence, for cooking fish to achieve a safe and palatable dish, the cooking method that causes the least physicochemical changes in fish should be applied, regardless of whether traditional cooking methods or emerging cooking methods, such as microwave cooking, are used. In the last decade, microwave cooking has become the most commonly used method for cooking food; hence, this cooking method was also included in the scope of the study.

Hence, the mentioned cooking methods not only increase the shelf life of fish by inactivating pathogenic microorganisms but also enhance its flavor and taste (Ersoy and Özeren, 2009). In specific, processing methods (e.g., boiling, steaming, frying, and microwaving), time and temperature are significant prevailing factors that denature myofibrillar protein (myosin and actin), resulting in muscle toughness and juice loss in fish (Chiavaro et al.2009). Water retention is one of the major factors determining fish texture; it resulted in improved water content in muscle tissues and decreased mechanical strength (Domínguez et al. 2014; Hultmann and Rustad 2002). The quantity of collagen and lipids also play a major role in texture composition; they influence firmness, palatability, and juiciness, which are interrelated to water retention during the cooking process (Fallah et al. 2011; Joo et al.2013). In addition to these properties, the color properties of cooked fish are important criteria and provide consistent evidence about the eating quality attribute (Garcí et al.2007). In general, fish are very susceptible to oxidation due to their complex chemical and physical composition. Similarly, *Labeo bata* is also susceptible to oxidation. Many studies have determined the antioxidant capacity of fish through extraction-based methodologies involving different solvents (Najafian and Babji, 2012; Tang, et al. 2001; Wu et al. 2003). However, these methodologies measure only the antioxidant capacity of the extracted soluble hydrophilic and hydrophobic fraction; moreover, these solvents do not facilitate complete fractionation. Therefore, in this study, the QUick, Easy, New, CHEap and Reproducible (QUENCHER) technique was employed to analyze the total antioxidant capacity (TAC) of raw and cooked *Labeo bata* fillets (Serpen et al. 2012). This study investigated the influence of different cooking procedures on the chemical composition, mineral content, amino acids profile, fatty acid profile, physico-chemical characteristics and in-vitro antioxidant activities of Indian mackerel.

## Materials and methods

### Samples preparation

*Labeo bata* (n = 20) were procured from the ukkadam fish market in coimbatore, India. The average weight and length of *Labeo bata* was 14.05+1.20 g and 20.9 ± 1.23 cm, respectively. Fish samples were washed with distilled water, dark muscle was eviscerated and filleted. The chemical composition, mineral content, fatty acid profile and in-vitro antioxidant activities were analyzed in the fish fillets only.

### Cooking process

Fish fillets were categorized into five groups based on the cooking process used. Four groups of fillets were subjected to different cooking processes, namely boiling (100 °C for 10 min), steaming (100 °C for 10 min), microwaving (2450 MHz for 6 min), and frying (180 °C for 5 min) by using the AOAC standard method and raw fish fillets was used as control. After cooking the fish fillets were placed for 10 min on absorbent paper towels and

endpoint temperature measurements for each method were evaluated for each cooked fish fillets samples (Data not shown). Refined sunflower oil was the medium used for frying. After accomplishment of the cooking process, fish fillets were cooled to room temperature, and their skin and backbone were removed. For the characterization of proximate composition and physico-chemical properties, all fish fillets were homogenized in a food blender (HR1363 600-W Hand Blender, Bangalore, India). Furthermore, for the assessment of Fourier transform infrared (FT-IR) spectra and TAC, cooked samples were freeze-dried. All fish fillets homogenates were assayed in triplicate.

**Proximate analysis**

Proximate compositions (ash, fat, protein, and moisture contents) of processed and control fish fillets were analyzed according to AOAC standard procedures (AOAC, 1996).

**Spectrum analysis**

The FT-IR spectra of processed and control fish fillets were recorded using an FT-IR spectrometer (Thermo Nicolet Model: 6700, UK) at 25 °C. The transmission mode for spectrum analysis of samples was recorded within the range of 500 to 4000 $cm^{-1}$ and the KBr pellet used as a blank.

**Nutritional composition**

**Amino acids composition**

The amino acid composition of raw and cooked *Labeo bata* was analyzed based on the standard procedure of AOAC method (2002). Three different hydrolytic methods were used to determine. (i). Acid hydrolysis using 6M HCl was first completed to derive amino acids other than methionine, cysteine, and tryptophan (ii).Performic acid hydrolysis was used to obtain sulfur containing amino acids such as methionine and cysteine, (iii). Alkaline hydrolysis using 4.3N LiOH was used to obtain tryptophan. Samples were cooled and diluted to the < 13 µmol ml-1 using water and 0.25µmol ml-1 internal standard DL-α amino butyric acid. Amino acid calibration standard (100µM) with the internal standard was prepared. Standard and samples were derivative with 6-aminoquinonyl-N-hydroxyl Succinimidyl Carbonate (AQC) using an AccQ- Flour reagent kit. Derivatised samples and standards (20µl) were injected into RP -LC equipped with AccQ Tag C18 column (3.9 mm X 150 mm). The mobile phase was two eluents A and B. AccQ-Tag eluent A was prepared with 140mm sodium acetate and 17 mm triethylamine titrated with phosphoric acid to pH 4.5, and eluent B was acetonitrile (0.6 g mL-1). The flow rate was set at 1 mL min-1, and the elution of amino acids was detected with a fluorescence detector.

**Fatty acid composition**

Fatty acids were isolated from processed and control fillets according to the technique described by Momenzadeh et al. (2017). Fatty acid methyl esters (FAME) of the extracts were analyzed using gas chromatography (Hewlett Packard, HP, Palo Alto, USA) prepared with a fused silica column (30 m × 0.25 mm diameter, 0.25 µm thicknesses), flame ionization detector (FID) and split or split less injector. The FID temperature was set at 240 °C and 280 °C respectively. The column temperature increased from 180 °C to 200 °C at 2 °C/min. FAMEs concentration was presented as a percentage of the sum of the particular fatty acids.

**Mineral composition**

Mineral analysis of processed and control fish fillets was extracted with acidic digestion by concentration $HNO_3$ (AOAC, 1996) and detection and quantification by ICP-AEC (Arcos, Spectro, Germany). All minerals were conveyed as mg/kg fish dry weight.

***In-vitro* antioxidant measurement**

**Preparation of processed and control fish fillets for QUENCHER procedure**

Following the procedure of Serpen et al. (2012) with slight necessary modifications, the TAC of raw and cooked fish fillets was analyzed using the QUNCHER procedure. For the analysis, the 1:5 (w/w) solid-state dilution of freeze-dried raw and cooked *Labeo bata* fillets was performed using cellulose. Over the 3-h observation period of the reaction, cellulose was observed to be inert when mixed with free radical solutions. TAC was assayed using free radical probe solutions such as ABTS+, DPPH, and FRAP.

**Preparation of ABTS solution**

ABTS stock (solution-A) was prepared by dissolving 38.41mg of ABTS in 5ml of deionized water. Potassium persulfate stock (solution-B) was prepared by dissolving 6.62 mg of potassium persulfate in 5 ml of deionized water. Thus, the prepared 10ml stock solution (5 ml solution A + solution B) comprised 7mmol/L ABTS and 2.45mmol/L potassium persulfate. Subsequently, the stock solution was allowed to stand in the dark for 16 h at room temperature. Working ABTS+ solution was freshly prepared by diluting the 10ml stock solution with 800ml of an ethanol–water mixture (50:50v/v) until its absorbance was 0.75–0.80 at 734nm.

**Preparation of DPPH solution**

DDPH stock solution was prepared by dissolving 20 mg of DPPH in 100 mL of ethanol. Furthermore, to prepare the working standard, this stock solution was diluted with 100 ml of deionized water to obtain a solution with an ethanol/water ratio of 50:50 (v/v) having an absorbance of 0.75–0.80 at 525nm.

**Preparation of Ferric ion reducing antioxidant power (FRAP) solution**

FRAP radical solution was prepared by dissolving 10mM TPTZ and 20mM ferric chloride in300mMsodium acetate (pH 3.6) at a 1:1:10 ratio, as described by Benzie and Strain (1996).

**Measurement of total antioxidant capacity**

TAC measurements of fillets were performed following the procedure of Serpen et al. (2012) with necessary modifications. In the QUENCHER procedure, cellulose-diluted, freeze-dried raw and differently cooked fish samples (~10.0 mg) were positioned in a centrifuge tube and reacted with 10 ml of each of ABTS+, DPPH, or FRAP working reagents for the ABTS, DPPH, and FRAP radical scavenging assays, respectively. The mixture was rigorously mixed for one min and incubated on an orbital shaker at 400 rpm in the dark at room temperature. The incubation periods were 30, 60, and 10 min for the ABTS, DPPH, and FRAP assays, respectively. Subsequently, centrifugation was performed at 9200 ×g for 3 min, and the clear supernatant was read for absorbance at 734, 525, and 734 nm for the ABTS, DPPH, and FRAPS assays, respectively. In the DPPH, FRAP, and ABTS assays, radical scavenging capacity was calculated in mmol of Trolox equivalent antioxidant capacity (TEAC)/kg fillet dry weight by using standard calibration curves.

**Physicochemical measurements**

**Cooking loss**

After cooking, the samples were cooled at 25 °C for 30 min, and the percentage of cooking loss was evaluated according to the standard AOAC method (1996).

**Water activity and pH**

The water activity ($a_w$) of samples was analyzed using a water activity meter (Aqualab Series 4TE, Decagon Devices, Inc., USA). Measurement of water activity was performed until the value was concurrent. The pH value of processed and control fish fillets was analyzed using a digital pH meter (MetrohmAG, Switzerland). The pH electrode was inserted directly into raw and cooked fish fillet at the center part of each sample according to the method of Manthey et al. (1988).

**Color analysis**

The color of processed and control fish fillets was read as *L**, *a**, and *b** using Lab Colorimeter (D-25, Hunter Associated Laboratory, USA). The chroma (C*) and hue angle (H°) values of samples were calculated by the formulas, $C^* = (a^{*2}+b^{*2})^{1/2}$ and $H° = \tan^{-1}(b^*/a^*)$, respectively.

**Texture profile analysis**

The texture profile analysis (TPA) of processed and control fish fillets were performed using a texture profile analyzer (HDP/BS, TA-HD plus, Stable Micro Systems, Surrey) was used to measure texture parameters like springiness cohesiveness, gumminess hardness, adhesiveness. For the analysis, samples with a surface area of 5×5 $cm^2$ were obtained from the epaxial part of fresh and cooked fish fillets. Textural properties were determined by analyzing two sequential compression tests with a cylindric shaped probe with a diameter of 30 mm separated by a rest phase of 60 s.

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**Statistical analysis**

All parameters of processed and control fish fillets were evaluated using one-way analysis of variance. Post hoc analysis was performed using Duncan's multiple range test. Statistical significance was set at 95%. All analyses were performed using SPSS, version 20.0 (IBM Corporation, Armonk, New York).

## Results and discussion

The differences in the proximate composition of fish fillets after the cooking process are presented in Table (1). There was a decrease from 74.6% to 63.5 % in the moisture content of *Labeo bata* fillets cooked using various cooking methods. Minimum moisture loss was observed for boiled fish fillets, whereas maximum moisture loss was noted for fried and microwaved fillets. Decrease in moisture content of samples may be associated to the applied temperature which can denature protein structure (disturbing the protein's ability to bond with water). Further, different temperatures can evaporate muscle water at different levels. Ash content of raw fish fillets was 1.3%, and the ash content of fish fillets cooked using various cooking methods varied. The highest ash content (1.7%) was recorded for fried fish fillets. Raw *Labeo bata* fillets comprised 18.8% protein. The results showed that having compared different cooking methods, it could be noted that the protein amount were the highest in microwave-cooked fillets (27.1%). This could be due to the high loss (17.5%) of water occurring during microwave cooking which causes protein concentration (19.2%) to increase. On the other hand, the nutrient changes depend also on the type of cooking style and particularly on the applied temperature. A similar tendency was also noted for the lipid content, which augmented from 4.5% (raw fillets) to 11.5% (fried fillets). The increase of lipid may be attributed to oil absorption during the cooking process; heat treatment, oil penetration is prominent after the partial loss of water through evaporation (Saguy and Dana, 2003). The substantial increase in ash, protein, and lipid contents in differently cooked fish fillets were correlated with the decrease in moisture content (Ersoy and Özeren, 2009). Rosa et al., (2007) also reported the same amount of fat, protein and ash contents of cooked samples increased in comparison with the uncooked samples due to the increase in the concentration effect caused by moisture loss.

**Table 1.** Proximate (%) and mineral composition (mg/kg) of raw and processed *labeo bata*

| Parameter | Types of Cooking | | | | |
|---|---|---|---|---|---|
| | Raw | Boiled | Steamed | Fried | Micro-waved |
| Moisture (%) | 78.6 ± 0.62[a] | 70.2 ± 0.57[b] | 68.6 ± 0.59[b] | 63.5 ± 0.58[c] | 64.9 ± 0.26[c] |
| Protein (%) | 16.8 ± 0.02[e] | 17.5 ± 0.03[d] | 19.7 ± 0.05[c] | 20.9 ± 0.04[b] | 21.1 ± 0.01[a] |
| Lipid (%) | 4.5 ± 0.05[d] | 4.9 ± 0.08[c] | 5.3 ± 0.05[c] | 11.5 ± 0.48[a] | 6.5 ± 0.31[b] |
| Ash (%) | 1.3 ± 0.01[e] | 1.5 ± 0.11[d] | 1.5 ± 0.12[c] | 1.7 ± 0.04[a] | 1.6 ± 0.01[b] |
| ***Mineral composition*** | | | | | |
| Na | 68.3± 1.32[d] | 65.2 ± 0.98[e] | 85.2 ± 1.56[c] | 92.2 ± 1.08[b] | 95.3 ± 0.87[a] |
| K | 1243.0 ± 3.21[e] | 1275.3 ± 2.56[d] | 1312.3 ± 3.01[c] | 1346.7 ± 2.56[b] | 1358.2 ± 3.08[a] |
| Ca | 147.2 ± 1.56[d] | 154.3 ± 1.98[c] | 215.4 ± 1.47[c] | 258.1 ± 0.97[a] | 231.8 ± 1.93[b] |
| Mg | 28.1 ± 0.08[e] | 31.0± 0.18[d] | 42.7 ± 0.25[a] | 38.5 ± 0.32[b] | 40.3 ± 0.45[b] |
| Fe | 2.3 ± 0.02[d] | 1.8 ± 0.04[e] | 3.5 ± 0.08[b] | 3.4 ± 0.03[c] | 3.8 ± 0.04[a] |
| P | 205.2 ± 2.42[e] | 212.6 ± 2.56[d] | 350.4 ± 2.98[a] | 346.3 ± 3.05[b] | 315.1 ± 3.56[c] |
| Mn | 0.77 ± 0.02[a] | 0.72 ± 0.01[b] | 0.58 ± 0.03[e] | 0.68 ± 0.01[c] | 0.66 ± 0.01[d] |
| Cu | 1.3 ±0.06[e] | 1.5 ± 0.03[c] | 1.4 ± 0.07[d] | 2.8 ± 0.02[a] | 1.8 ± 0.03[b] |
| Zn | 5.7 ± 0.04[a] | 4.2 ± 0.07[c] | 5.28 ± 0.18[a] | 5.2 ± 0.62[a] | 5.4 ± 0.56[a] |

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same column have no common superscripts are significantly different ($p$<0.05).

**Structural measurements**

The FT-IR spectra of differently cooked *Labeo bata* are presented in Fig. (1). FT-IR spectroscopic analysis was performed to identify the changes in the functional groups of the samples under study. The samples were scanned in the range from 500cm$^{-1}$ to 4000cm$^{-1}$. The majority of changes were observed in the region of 3600–1000 cm$^{-1}$. The bands centered at 3435.16 (boiled), 3340.24 (fried), 3345.94 (microwave), 3435.16(raw), and 3452.13 (steamed) may correspond to the presence of glyceride ester carbonyl (Gomez et. al 2007). The weak bands found around 3300 cm$^{-1}$ may correspond to the presence of hydroxyl groups (peroxides and hydroperoxides) following oxidation. The peaks present between 2800–3000 cm$^{-1}$were caused by the asymmetric and symmetric stretching vibrations of methyl groups (Bertram et al. 2006). The bands present at 1616.12 (boiled), 1619.98 (fried), 1648.86 (microwave), 1652.75 (raw), and 1614.19 (steamed) were attributed to the stretching vibrations of carbon–carbon double bonds or conjugated double bonds. The bands present between 1300 and 1400 cm$^{-1}$ corresponded to the deformation vibrations of $CH_2$ (methylene) groups of unsaturated fatty acids (Guillén and Cabo, 1999). The bands present around 1100cm$^{-1}$ may be ascribed to the presence of saturated acyl groups of lipids (Bertram et al. 2006). The variation in the transmittance intensity of bands may be explained on the basis of the formation of oxidation products (primary and secondary).

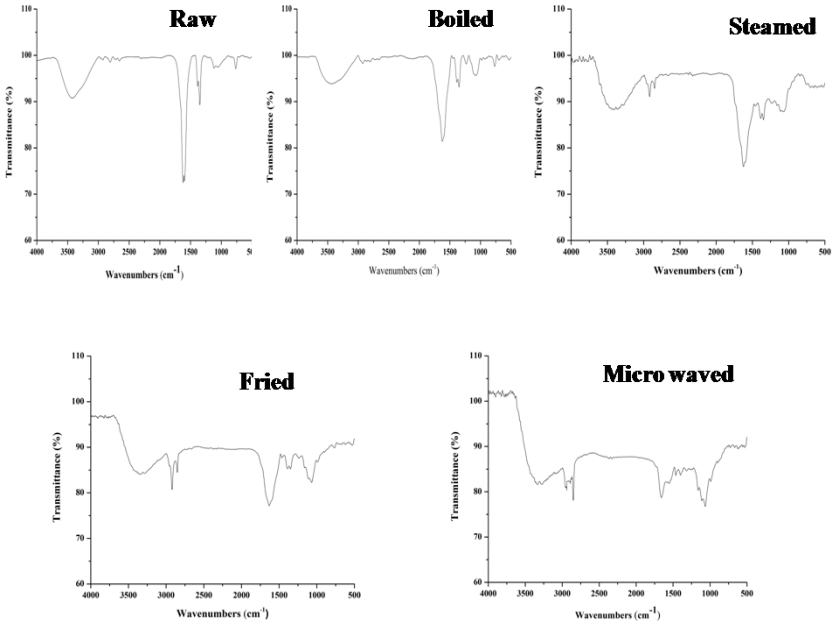


**Fig. 1.** FT-IR spectra of raw and processed *labeo bata***.**

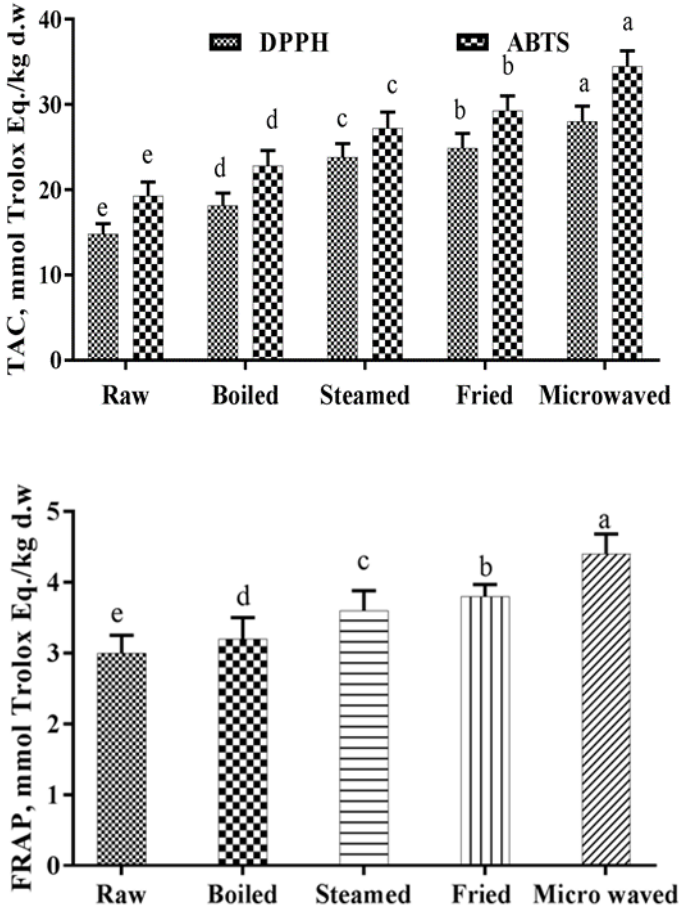


**Fig. 2.** A). TAC of raw and processed *labeo bata* evaluated using DPPH and ABTS as antioxidant probes and b) FRAP of raw and processed *labeo bata* (mean ± standard deviation)

**Nutritional measurements**

**Amino acids profile**

Table 2 shows the amino acid composition of *Labeo bata* prepared from the different cooking method. The quantity and proportions percentage of total amino acids significantly improve in all the cooking methods. The amino acid composition is important in the therapeutic process, and its composition in fresh water fish foods is similar to that in human beings diet. Therefore, consumption of fresh water fish foods acquires the essential and nonessential amino acids in large quantity and proper balance in the diet (Osibona et al. 2009). The results note that the essential amino acids (EAA) and nonessential amino acid (NEAA) content of microwaved and fried sample were found to be significantly higher than that of boiled and steamed sample *($p<0.05$).* The total essential amino acids value varied from 723.28 and 964.06 mg/g of cooked samples. Processed fishes have been reported to have an abundant quantity of essential amino acids. The previous studies have been reported to exhibit variation in their amino acid composition of cooking methods (Oluwaniyi et al. 2010; Ismail et al. 2004) which depends on several factors such as duration of time and cooking temperature and non- availability of the amino acids in the raw material (Burger and Walters, 1973).

**Table 2.** Amino acids composition of raw and processed *labeo bata* (mg/100g)

| Amino acid | Raw | Boiled | Streamed | Fried | Micro waved |
|---|---|---|---|---|---|
| Lysine | 256.78±8.35$^{d}$ | 289.28±9.02$^{c}$ | 287.79±10.66$^{c}$ | 307.58±12.06$^{b}$ | 319.67±9.52$^{a}$ |
| Methionine | 307.37±13.2$^{d}$ | 315.34±12.2$^{c}$ | 304.49±9.53$^{d}$ | 326.57±10.1$^{b}$ | 335.45±10.7$^{a}$ |
| Threonine | 141.89±13.7$^{b}$ | 143.23±14.3$^{b}$ | 142.97±15.2$^{b}$ | 153.78±14.3$^{a}$ | 155.2±14.6$^{a}$ |
| Isoleucine | 202.02±12.8$^{e}$ | 214.51±19.0$^{c}$ | 206.17±15.5$^{d}$ | 222.34±15.1$^{b}$ | 226.1±14.2$^{a}$ |
| Leucine | 286.60±16.2$^{c}$ | 310.67±17.4$^{b}$ | 293.82±13.2$^{c}$ | 318.17±13.1$^{ab}$ | 325.8±11.1$^{a}$ |
| Phenylalanine | 302.28±10.2$^{d}$ | 307.59±16.6$^{c}$ | 297.15±19.6$^{e}$ | 317.09±12.2$^{b}$ | 324.3±16.5$^{a}$ |
| Valine | 250.92±14.6$^{d}$ | 253.16±18.2$^{c}$ | 243.63±19.1$^{e}$ | 260.94±16.2$^{b}$ | 267.3±10.7$^{a}$ |
| Histidine | 190.71±14.6$^{d}$ | 198.48±10.4$^{c}$ | 189.03±12.4$^{e}$ | 204.31±13.4$^{b}$ | 208.1±12.4$^{a}$ |
| Serine | 149.03±12.9$^{e}$ | 157.5±10.6$^{c}$ | 152.34±16.3$^{d}$ | 162.7±15.4$^{b}$ | 166.1±12.2$^{a}$ |
| Arginine | 147.92±16.0$^{d}$ | 158.7±16.4$^{c}$ | 151.91±12.4$^{d}$ | 163.95±15.6$^{b}$ | 166.4±13.4$^{a}$ |
| Cysteine | 140.67±15.7$^{d}$ | 148.2±16.1$^{c}$ | 141.67±12.3$^{d}$ | 154.66±13.0$^{b}$ | 156.7±16.7$^{a}$ |
| Aspartic acid | 302.34±10.3$^{d}$ | 311.66±13.4$^{c}$ | 296.62±10.4$^{e}$ | 319.66±10.1$^{b}$ | 325.2±10.9$^{a}$ |
| Glutamic acid | 486.46±11.6$^{d}$ | 495.56±13.7$^{c}$ | 478.20±16.4$^{e}$ | 513.32±10.6$^{b}$ | 524.4±17.6$^{a}$ |
| Alanine | 108.82±12.1$^{a}$ | 118.45±10.5$^{a}$ | 119.28±13.5$^{a}$ | 122.50±10.6$^{a}$ | 126.4±10.9$^{a}$ |
| Tyrosine | 156.58±12.1$^{a}$ | 146.74±10.4$^{c}$ | 144.79±12.9$^{c}$ | 151.28±10.5$^{b}$ | 155.9±15.6$^{a}$ |
| Glycine | 204.83±14.63$^{b}$ | 311.40±12.2$^{a}$ | 297.23±10.7$^{a}$ | 321.55±10.6$^{a}$ | 327.7±11.3$^{a}$ |
| Proline | 296.63±10.5$^{d}$ | 305.63±10.2$^{c}$ | 291.57±10.5$^{e}$ | 315.75±16.3$^{b}$ | 321.5±15.3$^{a}$ |

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same column have no common superscripts are significantly different ($p<0.05$).

**Fatty acids profile**

Table 3 displays the fatty acid profile in *Labeo bata* fish. Total abundant fatty observed in raw and cooked *Labeo bata* fish fillets were ranged as follows: 35 to 44 % saturated fatty acids (SFA), 23 to 24 % monounsaturated fatty acids (MUFA), and 18 to 23 % polyunsaturated fatty acids (PUFA). From the results, the individual fatty acid composition varied substantially between raw and cooked fillets. Palmitic acid (C16:0), oleic acid (C18:1n-9c), stearic acid (C18:0) and docosahexaenoic acid (DHA, C22:6n-3) were observed as most ample fatty acids in raw *Labeo bata* fish fillets. Fatty acid contents obtained in raw fillets (with 4.5 % total fat) followed a comparative pattern with SFA > MUFA > PUFA. These findings were moderately related to the fatty acid composition obtained for red mullet fish reported by Koubaa, Mihoubi, Abdelmouleh, and Bouain (2012). While, it was found that saturated fatty acids represents the major part of total fatty acids in *Labeo bata* primarily palmitic (C16:0) and stearic

(C18:0) acids (Gladyshev et al. 2006). As shown in Table 2, various cooking methods had significant effect on the total SFA (reduced from 44.56% to 35.66%). Whereas it was found that the cooking methods significantly increased the quantity of the total PUFA from 18.86% to 23.02%, but none of the cooking methods had a significant effect on the total MUFA. In general, the ratio of n-3/n-6 were used with various biomedical indexes (Weber et al. 2008). As shown in Table 2, there was no significant difference in ratio of n-3/n-6 of fillets between raw and cooked fish fillets, except steamed fish fillet (0.81). Among cooking methods, microwave (10.87%) and frying (10.65%) significantly increased the eicosapentaenoic acid (EPA) and DHA content. The changes in the fatty acid profile of the fish fillets during various cooking treatments are a significance of the water loss and induced of the heat treatment. Conversely, fried fish fillets showed huge changes in the fatty acid profile when compared to control (raw samples), perhaps due to concurrent oil absorption and moisture loss happening during the frying process. Garćıa- Arias et al. (2007) reported the same results that changes of fatty acid profile enhanced the value of various cooking methods, which depends on the composition of the frying oil and were not homogenous for the different fatty acids because some fatty acids decreased, while others increased. The recommended dietary intake of EPA+ DHA is about 200 and 500 mg per day (Harris et al. 2008). The results suggest that cooking method had improved the amount of EPA+DHA, while raw fillet had a comparatively low nutritive value, and hence cooking method cover recommended daily intake.

**Table 3.** Fatty acid composition (%) of raw and processed *labeo bata*.

| Parameter | Types of Cooking | | | | |
|---|---|---|---|---|---|
| | Raw | Boiled | Steamed | Fried | Micro-waved |
| C14:0 | 1.28 ± 0.02$^{a}$ | 0.85 ± 0.03$^{e}$ | 0.94 ± 0.02$^{d}$ | 1.08 ± 0.02$^{b}$ | 0.98 ± 0.04$^{c}$ |
| C16:0 | 30.68 ± 1.23$^{a}$ | 30.12 ± 1.05$^{a}$ | 30.19 ± 1.32$^{a}$ | 28.64 ± 1.08$^{b}$ | 28.12 ± 0. 98$^{b}$ |
| C18:0 | 12.6 ±0.98$^{a}$ | 7.04 ± 0.15$^{d}$ | 7.85 ± 0.28$^{b}$ | 7.92 ± 0.18$^{c}$ | 6.56 ± 0.27$^{e}$ |
| ∑SFA | 44.56 ± 1.30$^{a}$ | 38.01 ± 1.89$^{b}$ | 38.98 ± 1.45$^{b}$ | 37.64 ± 1.24$^{c}$ | 35.66 ±1.63$^{d}$ |
| C16:1 | 7.18 ± 0.56$^{c}$ | 6.92 ± 0.58$^{e}$ | 7.06 ± 0.45$^{d}$ | 8.56 ± 0.17$^{a}$ | 8.26 ± 0.86$^{b}$ |
| C18:1n-9c | 12.95 ± 0.98$^{b}$ | 12.98 ± 0.85$^{b}$ | 13.46 ± 1.02$^{a}$ | 11.65 ± 0.91$^{c}$ | 11.56 ±0.87$^{c}$ |
| C20:1 | 3.17 ± 0.03$^{e}$ | 3.21 ± 0.05$^{d}$ | 3.56 ± 0.07$^{b}$ | 3.98 ± 0.02$^{a}$ | 3.49± 0.08$^{c}$ |
| ∑MUFA | 23.3 ± 0.25$^{b}$ | 23.11 ± 0.10$^{b}$ | 24.08 ± 0.18$^{b}$ | 24.19 ±0.02$^{a}$ | 23.31 ±1.12$^{b}$ |
| C18:2n-6c | 0.69 ± 0.03$^{c}$ | 0.59 ±0.02$^{e}$ | 0.65 ±0.05$^{d}$ | 0.82 ±0.08$^{a}$ | 0.78 ±0.04$^{b}$ |
| C18:3n-6 | 0.38 ± 0.05$^{d}$ | 0.36 ±0.04$^{e}$ | 0.42 ±0.03$^{c}$ | 0.46 ±0.05$^{b}$ | 0.48 ±0.02$^{a}$ |
| C18:3n-3 | 0.29 ± 0.03$^{e}$ | 0.32 ±0.05$^{d}$ | 0.33 ±0.02$^{c}$ | 0.35 ±0.06$^{a}$ | 0.34 ±0.04$^{b}$ |
| C20:2 | 0.32 ± 0.02$^{a}$ | 0.28 ±0.05$^{d}$ | 0.29 ± 0.04$^{c}$ | 0.32 ±0.02$^{a}$ | 0.31 ±0.05$^{b}$ |
| C20:3n-6 | 3.65 ± 0.12$^{e}$ | 3.96 ±0.17$^{d}$ | 4.23 ±0.09$^{c}$ | 4.56 ± 0.12$^{a}$ | 4.45 ±0.18$^{b}$ |
| C20:4n-6 | 4.60 ±0.65$^{d}$ | 5.63 ±0.87$^{c}$ | 5.98 ±1.03$^{a}$ | 5.87 ±1.08$^{b}$ | 5.87 ±0.98$^{b}$ |
| C20:5n-3 | 3.56 ± 0.12$^{c}$ | 3.85 ± 0.18$^{b}$ | 3.45 ±0.14$^{d}$ | 4.78 ±0.13$^{a}$ | 4.89 ±0.16$^{a}$ |
| C22:6n-3 | 5.37 ± 0.45$^{b}$ | 5.32 ± 0.65$^{b}$ | 5.46 ±0.52$^{c}$ | 5.87 ±0.67$^{a}$ | 5.98 ±0.34$^{a}$ |
| ∑PUFA | 18.86 ± 1.03$^{c}$ | 20.31 ±1.04$^{b}$ | 20.79 ± 1.08$^{b}$ | 23.01 ±1.06$^{a}$ | 23.02 ±1.07$^{a}$ |
| ∑n-3 | 9.22 ± 0.10$^{d}$ | 9.49 ± 0.09$^{c}$ | 9.24 ± 0.08$^{d}$ | 11.0 ± 0.09$^{b}$ | 11.21 ± 0.10$^{a}$ |
| ∑n-6 | 9.32 ± 0.08$^{e}$ | 10.54 ± 0.10$^{d}$ | 11.28 ± 0.13$^{c}$ | 11.71 ± 0.06$^{a}$ | 11.58 ± 0.09$^{b}$ |
| n-3/n-6 | 0.98 ± 0.04$^{a}$ | 0.90 ± 0.02$^{a}$ | 0.81 ± 0.05$^{b}$ | 0.93 ± 0.03$^{a}$ | 0.96 ± 0.02$^{a}$ |
| EPA+DHA | 8.93 ± 0.02$^{d}$ | 9.17 ± 0.03$^{c}$ | 8.91 ± 0.05$^{d}$ | 10.65 ± 0.02$^{b}$ | 10.87 ± 0.04$^{a}$ |

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same column have no common superscripts are significantly different ($p$<0.05).

**Mineral analysis**

Table 1 displays the mineral content of *Labeo bata* influenced by different cooking methods. The content of minerals such as Na, K, P, Ca, Mg, Fe, and Cu of *Labeo bata* fillets cooked utilizing different cooking methods significantly (p<0.05) increased. The sodium content of the raw fillet was

noted as 68.3mg/kg. Increased Na level was noted in microwaved fillets, while a significant decrease was noted in boiled fillets. Potassium level (1358.2 mg/kg) was noted to be the highest in microwaved fillet, and raw fillets (1243 mg/kg) reported the lowest level. Highest levels of Ca (258.1 mg/kg) and Cu (2.8 mg/kg) were noticed in fried fish fillets, and the lowest value was observed in raw fish fillets. Significantly highest levels of Mg (42.7 mg/kg) and P (350.4 mg/kg) in steamed fillets and raw fillet recorded lowest Mg (28.1 mg/kg) and P (205.2 mg/kg). The manganese content of raw and cooked fillet varied from 0.58-0.77 mg/kg. In addition, significantly reduced Mn content was noticed in fish fillets by different methods of cooking. Lowest content of Mn (0.58 mg/kg) in steamed fillets and highest Mn level (0.77 mg/kg) was observed in raw fillets. Furthermore, the highest levels of Fe (3.8 mg/kg) and Zn (5.4 mg/kg) in microwaved fish fillets and lowest Fe (1.8 mg/kg) and Zn (4.2 mg/kg) content was noticed in boiled fish fillets. Changes in mineral composition with different cooking methods have been observed Hosseini et al. (2014) and reported similar findings in kutum roach fish. However, changes in mineral content of *Labeo bata* fish fillets by various cooking methods were comparable with previous studies reported (Gokoglu et al. 2004; Momenzadeh et al., 2017). The mineral content of *Labeo bata* was significantly improved during the cooking process in all methods employed. The Minerals levels was the highest in micro waved samples followed by fried fish fillets and ; this can be attributed to the loss of water during processing (Microwave and frying) thereby causing mineral level and oil up-take avoiding other minerals release. Similarly, Gokoglu et al. (2004) mentioned that mineral content of boiled fish decreased significantly, those of grilled and microwave-cooked fish significantly increased.

***In-vitro* antioxidant measurement**

The TAC of raw and cooked *Labeo bata* fillets was evaluated. The results are presented in Fig. 2A, and 2B. Native antioxidant capacity is usually altered on processing. Furthermore, in complex heterogeneous food systems such as fish, the evaluation of TAC through a single method is not possible because antioxidant capacity is attributed to different chemical mechanisms. In this study, three common radical probes, namely DPPH, ABTS, and FRAP, were used to evaluate the *in-vitro* antioxidant capacity of raw and cooked *Labeo bata* fillets. The DPPH radical scavenging assay is used to evaluate the hydrogen donating potency (Najafian & Babji A S 2018). By contrast, the ABTS radical assay is used to evaluate the single-electron transfer capability. In the FRAP assay, the $Fe^{3+}$ probe is used to evaluate the reductive antioxidant power (Benzie and Strain, 1996). Radical scavenging capacity is expressed in mmol of Trolox Eq/kg of fish (dry basis). Raw *Labeo bata* fillets showed the lowest FRAP value (3.0 ± 0.08 mmol Trolox Eq/kg (dw)). Statistically significant differences were observed in the FRAP values among differently cooked *Labeo bata* fillets. Highest FRAP values were noted for microwaved fish fillets (4.4 ± 0.09 mmol Trolox Eq/kg (dw)). Because the FRAP method is based on the reduction of the $Fe^{3+}$ TPTZ complex to the ferrous form at low pH, the lower FRAP values observed in raw fish may be due to the low availability of antioxidants for reducing the ferric form to the ferrous form. The total ABTS scavenging capacity of raw *Labeo bata* fillets was 19.3–34.5 mmol Trolox Eq/kg (dw) % inhibition. In the ABTS assay, the TAC values of differently cooked fish fillets were significantly higher than those of raw fish fillets. Proteins and peptides have an important role in contributing to the antioxidant capacity of fish. Protein has the ability to scavenge free radicals and chelate prooxidative metals. The ABTS scavenging capacity of cooked *Labeo bata* fillets was higher than that of raw fish fillets. In particular, microwaved

fish fillets showed the highest capacity. This finding may be attributed to peptides and the partial denaturation of protein in Indian mackerel.

The DPPH scavenging capacity of raw *Labeo bata* fillets was only 14.8 ± 0.20, whereas the microwaved sample showed the highest DPPH capacity (28.0 ± 0.24). When the TAC value determined from the ABTS and DPPH radical scavenging antioxidant assays was compared, significant differences were observed in TAC among differently cooked fish fillets. This finding may be attributed to the different radical scavenging abilities of the various antioxidant groups present in differently cooked fish fillets. In general, the reductive antioxidant capacity of microwaved *Labeo bata* fillets proved to be the highest in the ABTS and DPPH scavenging assays. This finding may be because of heating, protein denaturation results in the alteration of the tertiary and secondary structures of protein. The unfolding of protein and the formation of new peptides may increase the radical scavenging ability of the antioxidant groups.

**Physicochemical measurements**

**Cooking loss**

The cooking loss for differently cooked fish fillets is presented in Table 4. The cooking loss is directly dependent on the cooking process. Microwaved fish fillets showed significant ($p<0.05$) loss (35.5%), followed by fried (33.9%) and boiled fish fillets (26.6%). Minimum loss was noted for steamed fish fillets (24.9%). Boiled fish fillets showed a comparably low cooking loss. Significant loss of matter was correlated with higher cooking loss and was found to be linearly related to cooking temperature and time. Further mass transfer with thermal variation may have also affected water loss (Chiavaro et al. 2009). Water loss may be attributed to protein denaturation during the cooking process; protein denaturation may result in the low entrapment of water into the protein structure (Aaslyng, et al. 2003). Boiled fish fillets showed a comparably low cooking loss. This finding may be attributed to the presence of steam in the steam pot, which may have suppressed the evaporation of water from the fillet.

**Table 4.** Color and physical properties of raw and processed *labeo bata*

| Parameters | Types of cooking | | | | |
|---|---|---|---|---|---|
| | Raw | Boiled | Steamed | Fried | Micro-waved |
| Chromaticity | $10.5 \pm 0.06^{e}$ | $15.4 \pm 0.05^{c}$ | $16.0 \pm 0.01^{b}$ | $12.4 \pm 0.08^{d}$ | $16.8 \pm 0.057^{a}$ |
| Hue Angle (°) | $80.4 \pm 0.17^{e}$ | $85.3 \pm 0.37^{b}$ | $85.8 \pm 0.06^{a}$ | $81.7 \pm 0.12^{d}$ | $82.6 \pm 0.56^{c}$ |
| *L** | $71.1 \pm 0.85^{a}$ | $68.8 \pm 0.28^{b}$ | $64.3 \pm 0.67^{c}$ | $54.1 \pm 1.15^{d}$ | $46.5 \pm 1.49^{e}$ |
| *a** | $1.7 \pm 0.12^{b}$ | $1.2 \pm 0.08^{c}$ | $1.1 \pm 0.08^{c}$ | $1.8 \pm 0.18^{b}$ | $2.1 \pm 0.11^{a}$ |
| *b** | $10.4 \pm 0.18^{d}$ | $15.4 \pm 0.25^{b}$ | $16.0 \pm 0.09^{ab}$ | $12.5 \pm 0.09^{c}$ | $16.6 \pm 0.64^{a}$ |
| Cooking loss (%) | - | $26.6 \pm 0.39^{c}$ | $24.9 \pm 0.16^{d}$ | $33.9 \pm 0.29^{b}$ | $35.5 \pm 0.52^{a}$ |
| pH | $6.53 \pm 0.08^{d}$ | $6.39 \pm 0.47^{c}$ | $6.37 \pm 0.03^{c}$ | $6.26 \pm 0.05^{b}$ | $6.15 \pm 0.05^{a}$ |
| Water activity | $0.92 \pm 0.02^{a}$ | $0.88 \pm 0.08^{b}$ | $0.85 \pm 0.08^{c}$ | $0.74 \pm 0.05^{e}$ | $0.77 \pm 0.08^{d}$ |

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same column have no common superscripts are significantly different ($p<0.05$).WHC, water holding capacity.

**Water activity and pH**

Water activity ($a_w$) precisely measures the shelf life of any food system. This intrinsic property denotes the availability of free water in the food system. Reduced $a_w$ of a food system provides a shield to microbial growth and delays deterioration in the biochemical reaction (Primo-Martín et al., 2010). In a food system, moisture migration between domains can be avoided by adding an edible layer between the domains, resulting in a change in the water activity of the food ingredients (Suriya et al., 2017).By contrast, increased $a_w$ decreases the shelf life. In the current study, $a_w$ values of differently cooked fish fillets ranged from 0.77 to 0.88 (Table 4). The pH value of raw and differently cooked fish fillets was

summarized in Table 4. Rathod and Pagarkar, (2013) reported that the pH value of fish muscle layer is frequently a good list for quality assessment. It is significantly determining

fish quality as texture of fish. The pH value of raw fish fillets was 6.53. Raw fish fillets exhibited higher pH values than cooked fish fillets. The decrease in pH was not significant among boiled and steamed fish fillets, whereas microwaved fish fillets showed the lowest pH of 6.15. The pH value ranged between 6.8 and 7 was expectations as acceptance limit of fish and range above 7 were considered to be contaminated or spoiled (Kose et al., 2006; Orak and Kayisoglu, 2008).

**Color analysis**

The color characteristics of raw and cooked fillets are presented in Table 4. The color of raw and differently processed fish fillets was read as L* (lightness), a*(redness), and b* (yellowness) values. Moreover, these values were used to calculate color properties such as chromaticity and the Hue angle. Raw fish fillets exhibited the highest L* value, followed by boiled (68.8%) and steamed (64.3%) fish fillets. The lowest L* value was noted for microwaved samples. Furthermore, a* and b* values varied meaningfully from 1.1 to 2.1 and from 10.4 to 16.6, respectively. Differently cooked *Labeo bata* fillets showed significant changes in the b* value when compared with that (10.4) of raw fish fillets. The b* value was the highest for microwaved fish fillets. The L* value of differently cooked *Labeo bata* fillets significantly decreased. This finding may be correlated to protein aggregation, leading to increased opacity, low light transmittance, and the formation of colored compounds involving hydrogen sulfide, which are released from amino acids in the Maillard reaction (Hakimeh et al. 2010). Chromaticity was calculated as chroma (C*), which symbolizes the fullness of color. Microwaved fish fillets exhibited the maximum chromaticity. The hue angle denotes the sensitivity of color. From the findings, the hue angle (0 - 90°) of raw and cooked fish fillets was found to lie in the first quadrant, consistent with the sort of reddish-purple to yellow.

**Texture profile analysis**

Table 5 presents the effect of different cooking methods on the texture profile characteristics of *Labeo bata* fillets as compared with those of raw *Labeo bata* fillets. Cooking of raw fish fillets through different methods was observed to ($p \leq 0.05$) increase their hardness, springiness, gumminess, chewiness, and resilience significantly. Among the cooking methods, boiling caused lower increases in hardness, springiness, gumminess, and chewiness, whereas microwave cooking caused greater increases in these characteristics. The cohesiveness of differently cooked fish fillets was similar to that of raw fish fillets.

**Table 5.** Texture parameters of raw and processed *labeo bata*

| Parameters | Types of cooking | | | | |
|---|---|---|---|---|---|
| | Raw | Boiled | Steamed | Fried | Micro-waved |
| Hardness (g) | $27.6 \pm 0.32^{e}$ | $65.2 \pm 0.23^{d}$ | $78.4 \pm 0.52^{c}$ | $82.9 \pm 0.08^{b}$ | $88.5 \pm 0.15^{a}$ |
| Springiness | $0.33 \pm 0.05^{b}$ | $0.25 \pm 0.08^{d}$ | $0.29 \pm 0.03^{c}$ | $0.34 \pm 0.02^{b}$ | $0.41 \pm 0.03^{a}$ |
| Cohesiveness | $0.30 \pm 0.04^{a}$ | $0.31 \pm 0.08^{a}$ | $0.32 \pm 0.03^{a}$ | $0.31 \pm 0.03^{a}$ | $0.31 \pm 0.03^{a}$ |
| Gumminess | $8.57 \pm 0.10^{e}$ | $20.3 \pm 0.07^{d}$ | $24.3 \pm 0.16^{c}$ | $25.6 \pm 0.08^{b}$ | $27.4 \pm 0.02^{a}$ |
| Chewiness | $1.5 \pm 0.03^{e}$ | $5.4 \pm 0.08^{d}$ | $6.5 \pm 0.12^{c}$ | $9.5 \pm 0.05^{a}$ | $7.3 \pm 0.13^{b}$ |
| Resilience | $0.35 \pm 0.05^{e}$ | $0.41 \pm 0.03^{b}$ | $0.42 \pm 0.05^{b}$ | $0.45 \pm 0.05^{a}$ | $0.42 \pm 0.03^{b}$ |

Data expressed as Mean ± Standard deviation of Triplicates. Means within the same column have no common superscripts are significantly different ($p<0.05$)

Generally, cooking method increases the palatable texture of fish on mild heating due to biochemical changes such as shrinkage of muscle with the release of water, coagulation of myofibrillar proteins on the surface of solid fish, and damage of

connective tissues to facilitate the shredding of the fillets for the delicious consumption of fish (Kong, et al.2008; Kong, et al. 2007). Hence, the texture of cooked fish is related to the extent of cooking loss, and protein denaturation depends on the time and temperature of heating. Ofstad, Kidman, and Hermans (1996) reported that cooking loss increased as the temperature increased from 30 °C to 60°C. Kong et al. (2007) also observed that cooking loss increased rapidly to 14% after salmon was heated for 5 min, and cooking loss further increased to 20% after salmon was heated for 2 h at 121 °C. In protein denaturation, collagen starts to denature at a temperature of approximately 30°C. Myosin and actin become insoluble at approximately 55°C. Consequently, the highest cooking loss is associated with the highest degree of protein denaturation in cooked fish with the hardest texture. In the present study, the variation in textural characteristics may be attributed to the variation in the time and temperature of heating and accordingly to the variation in cooking loss and protein denaturation in differently cooked fish fillets. Although boiling was conducted at 100°C, the time of heating (10 min) was shorter than that in other methods. Hence, boiling might cause lower cooking loss and protein denaturation than other cooking methods; thus, boiling might exert lower effects on the increase in hardness of fish fillets.

## Conclusions

A total of 30% of the world's impoverished population can be found in South Asia, also known as Global South. Hence, any scientific intervention impacting food consumption and nutritional absorption has a larger impact on humanity. The present study thus attempted to identify the optimal cooking method for *Labeo bata* among the available cooking methods. The optimal cooking method should preserve the nutrient content of fish and produce advantageous changes in its physicochemical properties and TAC. The results revealed that all cooking methods influenced the proximate composition, nutritional properties, physicochemical properties and TAC of Indian mackerel. Changes in the cooking loss, pH, water activity, and WHC were more prominent in cooked fillets. Regarding color, cooking methods increased chromaticity, but decreased lightness. TAC evaluated using the direct QUENCHER procedure significantly increased in fish fillets cooked using any of the mentioned cooking method. Regardless of the cooking method, the increase in TAC values was attributed to the protein denaturation; break down of endogenous antioxidants and accumulation of Maillard reaction products. The consumers of *Labeo bata* should be made aware of this finding. Moreover, there is huge scope for calibrating the finding with products and for promoting its acceptance among people of all age groups and with differential nutritional needs.

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## Conflict of interest

There is no conflict of interest based on the writers.

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