

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Preparations

The freshly slaughtered catfish was collected and placed in the ice box to the faculty Science and Technology (FST), USIM immediately. Catfish from 3 different places of earthen pond; Simpang Renggam (Johor), Sungai Buloh (Selangor), and Lanchang (Pahang) were caught as farm-raised catfish. For wild-caught catfish were caught from their natural habitat at Sungai Skudai, (Johor). During sample preparation, all fresh catfish were rinsed thoroughly under running water and the dorsal fillet of catfish samples was collected as shown in Figure 3.1 and stored at $-20\text{ }^{\circ}\text{C}$ for the determination of proximate composition, texture. The catfish fillets were grouped into three for each catfish origin as triplicate.

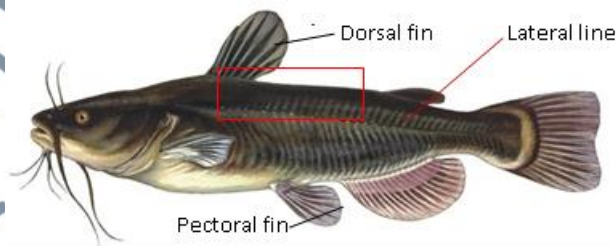


Figure 3.1: Dorsal Fillet Part of African Catfish Diagram

Table 3.1: Earthen Pond Condition

Pond Location	Pond Size	Catfish number	Feed type	Weed control	Feeding frequency	Pond system
Pond A (Lanchang, Pahang)	9.5m x 5.5m	150 fish/pond	Wheat bran pallet	No	Twice/day	Open
Pond B (Simpang Renggam, Johor)	4.5m x 1.2m x 1m	150 fish/pond	Chicken offal, chicken stomach	No	Twice/day	Close
Pond C (Sungai Buloh, Selangor)	6.5m x 1.4m x 0.8m	150 fish/pond	Fish pallet, vegeta bles	No	Twice/day	Close

3.2 Analysis

3.2.1 3.2.1 Chemical Composition Analysis

3.2.1.1 Moisture Content

The moisture content of catfish fillet was determined according to Association of Official Agricultural Chemists (AOAC) Official Method (Jr. Bradley & L. Robert, 2010). Approximately 5.0 g fillet was placed in a crucible and the fillet was dried in a previously heated oven at 105°C overnight. The weight of the sample without the crucible was calculated before and after drying as follows.

$$\text{Moisture (\%)} = \frac{w_1 - w_2}{w_1} \times 100$$

w₁ = weight (g) of sample before drying

w₂ = weight (g) of sample after drying

3.2.1.2 Ash Content

The ash content of catfish fillet was determined based on AOAC Official Method (Marshall, 2010). Approximately 5.0 g fillet is placed in a ceramic crucible in an oven at 130°C overnight. On an electric hot plate, the sample was burned until it stopped smoking. The charred sample was put above the crucible in a cold muffle oven and heated for 18 hours to 550°C. Final weight for ash was determined.

3.2.1.3 Fat Content

Fat content of catfish fillet was determined using a Soxtherm according to AOAC Official Method 948.22 (Min & Ellefson, 2010). A known weight of the fillet (approximately 10g/thimble) was defatted in a Soxtherm apparatus using petroleum ether (boiling point range = 38.2 - 54.3 °C) as the solvent (product-to-ratio of 1:10 w/v) for two hours. The remaining ether was evaporated, and the sample was dried at 105°C overnight (approximately 10-12 hours) in a fume hood. Fat content was then determined gravimetrically.

Total fat content (%) = [initial weight of full fat product (g) – final weight of defatted product (g)] / [initial weight of full fat product (g)] x100

3.2.1.4 Protein Content

Protein content of catfish fillet was determined according to AOAC Official Method 928.08 (Chang, 2010). Total proteins were determined using the micro-Kjeldahl technique by Kjeldahl distillation systems (Vapodest 450). In a micro-Kjeldahl flask, 2.0 g of fillet was inserted. A catalyst (mixture of 0.42 g of CuSO₄ + 9.0 g of

K₂SO₄), a few glass beads (to prevent sample bumping), and 15mL of concentrated H₂SO₄ was added to each sample. The sample was digested for 45-75 minutes at 410 °C (until a clear green solution is obtained, which ensures complete oxidation of all organic matter).

The digest was then diluted with 50 mL of distilled water before being attached to the distillation unit through the micro-Kjeldahl flask. Then, the sample was distilled by the addition of 45mL of 15N NaOH and released ammonia was transferred into a boric acid solution with methylene blue and methyl red (1:2). Borate anion was titrated with standardized 0.1 N of Sulphuric Acid (H₂SO₄) (in proportion to the amount of nitrogen). Meanwhile, a reagent blank was run. The crude protein content and nitrogen value in % was shown by the machine. The formula was used to determine the nitrogen (percent N) content of the sample.

$$\% \text{ N} = \frac{[(\text{mL of H}_2\text{SO}_4 \text{ for sample} - \text{mL of H}_2\text{SO}_4 \text{ for blank}) \times \text{standard-acid of H}_2\text{SO}_4 \text{ 0.1N or 0.05 mol/l} \times 1.4007]}{\text{weight of sample (g)}}$$

Protein % = total N (%) x approximate factor for sample (6.25).

3.2.2 3.2.2 Fatty acid composition

Fatty acid composition of catfish muscle was carried out according to method AOAC Official Method 996.06 and modified AOAC 996.06 (Gressler et al., 2010). 5g of fillet was weighed and 4mL of sodium methoxide (2N) is added. Then, the mixture is vortexed every 5 minutes for 20 minutes at room temperature. Next, 4 mL of a sulphuric acid solution was vortexed a few seconds in 50% of methanol before adding 2 mL of distilled water. 2.5 mL of hexane was used to extract the organic phase (which

contains fatty acids and methyl esters). The FAMEs were separated and quantified using a gas chromatograph equipped with a flame ionization detector.

The following were the chromatographic conditions: Initial column temperature 120 °C, maintained for 5 minutes, then set to rise at a rate of 5 °C/min up to 200 °C, maintained for 2 minutes, then at 1 °C/min up to 230 °C, maintained for 3 minutes. The injector and detector were maintained at 260 and 280 °C, respectively. The carrier gas was helium, with a constant flow rate of 1.1 mL/min and a column head pressure of

35.56 psi. A column RT-2560 with actual length 110m, 0.25 mmID width, and 0.20µm depth was used. A split ratio of 1:50 is used and 1 L of solution is injected. Individual FAMEs are recognized by comparing their retention periods to the retention times of verified standards (Supelco 37 component FAME Mix). Data regarding FAME composition were expressed in percentage according to the weight of the total identified FAMEs.

3.3 Fillet Texture Analysis

Fillet texture analysis was performed using a texture analyzer (TA.XT plus C) with some modification by using suitable probe for fish fillet sample (Basmal, 2021). The dorsal fillet samples of 4 catfish above the lateral line from each tank were filleted, packed in polyethylene bags with ice, the fillets were prepared into 1 cm × 1 cm × 0.5 cm blocks for texture detection of raw fillet and cooked fillet, respectively. The processing of cooked fillet was as follows: The cooking was performed in an oven at 180°C for 35 min (core temperature: 72°C) without fat or oil addition. Each sample of catfish was prepared for 3 replicates.

The fillet texture, including firmness, chewiness, gumminess, and cohesiveness. All the samples were measured by a texture analyzer. In short, the samples were compressed twice to 60% deformation with a flat-ended cylinder probe (8 mm diameter). The trigger force was 5g and the holding time was 5 seconds, meanwhile, the pre-test speed was 5 mm/s, and the post-test speed is 2 mm/s. The shearing force of fillets was measured by texture analyzer. Each sample was analyzed for triplicates.

3.4 Sensory Analysis

Sensory evaluation by trained panelists was recruited from the students of Food Biotechnology Program. The experiment conducted at Faculty of Science and Technology, Universiti Sains Islam Malaysia (USIM). Candidates underwent a screening process which includes sensory attributes training to determine their ability to recognize and name basic tastes and common aromatics in fish (Bechteal et al., 2018). The selected panel of 10 members ranged in age familiar with catfish as their meals were targeted. Panel training covers a period of 2 hours for 3 sessions. Panelists were educated in basic sensory descriptive analysis during the first and second sessions. The creation of descriptors for fish in general as well as wild-caught and farm-raised catfish were discussed during the final session training. Also, report by (Martine van der Ploeg, 1991) as reference for choosing the suitable sensory attribute in this study. Individually, panelists listed phrases that described the flavour notes they detected in the samples. Through guided conversations, unnecessary phrases were deleted, and agreements on sensory ballot terms were established.

Simple procedures such as analyzing the range of results for each characteristic, were used to evaluate panel performance throughout training. Individual mean scores

were compared to panel mean scores, and members who use restricted intensity scale ranges or have a high standard deviation in their evaluation of repeated samples were trained to enhance performance. The trained panelists were given samples and asked to describe and rate them based on factors such as taste/flavour, texture rating, scent, and appearance. Sensory evaluation features of catfish samples include odour, appearance, juiciness, texture, and flavour attributes as in Table 3.2.

Catfish fillet samples were processed before undergoing sensory evaluation. The processing of cooked fillet was as follows: The cooking was performed in an oven at 180°C for 35 min (core temperature: 72°C) without fat or oil addition. The cooked fillet was cut into 5cm x 3 cm size panelist plates.

Table 3.2: Sensory Attributes of Wild-caught and Farm-raised Catfish

Feature	Attribute	Definition
Odour	Grassy	Flavours that can be associated with the odours or flavours of leafy green vegetables and fresh cut grass.
	Rancid	Smell gross because of old or spoiled fat or oil.
	Muddy-earthly	Odour gives soil or mud that earthy smell and sometimes give wine or beets that earthy taste.
Appearance	Colour on top (Light-dark)	Light, white, or dark, yellowish colours.
	Underneath (Brown grey)	Dark brownish or light grey colour.
	Black threads	Black threads in flesh.
Juiciness	Moisture release (Juicy initial)	Bite with molars then evaluate the amount of liquid released when the sample is placed on tongue and pressed to the roof of the mouth.
	Moisture retention (Juicy midpoint)	Amount of liquid observed in the mass after 5 chews with the molar teeth.
	Pasty/Soft	Fish which sticks together like paste in the mouth when mixed with saliva. Forms a cohesive mass which may adhere to the soft tissue surfaces of the mouth or fingers.
Texture	Firm	The fillet is not soft or yielding when pressed and securely fixed in place. Exhibits moderate resistance when force is applied in the mouth or by touch.
	Fibrous	The perception of filaments or strands of muscle tissue during mastication.
	Flakiness	The ease of breaking the fish into small pieces with a fork.
Flavour	Charred	Partially burnt and carbonized taste.
	Nut-like	Acceptable sweet taste flavour of fresh fish.
	Metallic	Having a persistent metal, rancid, sour, or bitter taste.

(Source: Martine van der Ploeg, 1991; Peter B. Johnson, 1987)

3.5 Statistical Analysis

Wild-caught and farm-raised catfish raw data of proximate, and texture analysis from three different pond A, B, and C were analyzed by one-way analysis of variance (ANOVA) using Tukey's test to compare the means between samples. Data was analyzed using Minitab software, Release 18 and the statistically significant was established at ($P < 0.05$). All experiments were carried out in triplicate.

