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Nutritional and Microbiological Characteristics of Snakehead Fish

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Abstract
This study aimed to determine the physicochemical characteristics of the Snake Head Fish. Snakehead fish (*Channa Striata*) processing is divided into three types of supplements: original snakehead fish supplement (without any additional food), snakehead supplement which is varied with tofu drugs, and fish supplement which varied with turmeric extract. The results of proximate analysis showed that fish contains 63.69% of moisture, 0.31% of protein, 0.06% of fat, 10.97% of carbohydrates, and 0.01% of ash. In this study, 16 free amino were detected and glisin showed significantly the highest content (406.9 4± 3.62 mg/kg). Six minerals were quantified in fish (Na, K, Ca, Mg, Mn and P). The albumin content in snakehead fish was 1054.53 µg /g. The result of physical analysis showed that the particle size was 665.3 nm.

Keywords: Snakehead Fish, turmeric extract, albumin content and *Channa Striata*

1.0 INTRODUCTION

Channa striata, the striped snakehead, is a species of snakehead fish. It is also known as the common snakehead, chevron snakehead, or snakehead murrel and generally referred simply as mudfish. It is native to South and Southeast Asia, and has been introduced to some Pacific Islands.

The common snakehead (*Channa striata*) is a widely distributed tropical fish species from the Channidae. It is ecologically important as a major aquatic predator, known for its hardiness and adaptability, and prized as a food fish and sport fish. Occasionally, the common snakehead is kept as a "monster fish" in the ornamental trade, where like other snakeheads it is appreciated for its bold and active disposition. One of the foods that contain high albumin is snakehead fish. Snakehead fish (*Channa striata*) has a very high albumin fraction of protein compared to other animal protein sources, reaching more than 50% (R. M. Rosyidi *et al* 2019). The protein content of snakehead fish flour is included in the Quality or Grade I (good) category based on the quality standard of fish flour (Indonesian National Standard 01-2715-1996). EAAs (Essential amino acids) play a role in facilitating the gut microbiota in the gastrointestinal tract required for metabolic function and the immune system of the human beings (U. Grohmann *et al* 2017, F. Bifari *et al* 2017). The main aim of this paper is to accessibly summarise information on the biology of the common snakehead for the benefit of casual audiences such as nature enthusiasts, ornamental fish/aquarium hobbyists and students of biology. In addition, this page also includes a section on the taxonomy and systematics of the common snakehead for more technically-inclined readers.

The fish length was distributed from **23.0 to 65.0 cm, with an average of 33.0 cm**, although the monthly measurement majorly spread out at a range of 26.0–46.0 cm.

2.0 Materials and Methods

2.1 Sample Collection: -Snakehead fish (*Channa striata*) was obtained from traditional markets in Guntur. Its weight is between 600-900 g (medium size category). The selection of this weight is optimizing the yield and steaming process. Other material includes tofu dregs, and turmeric obtained from the traditional market in Guntur. The equipment used in snakehead fish flour processing is a blender, knife, water bath/heater, pan for steaming snakehead fish, oven, sieve, and a digital scale. Nutritional and microbiological characteristics use analysis services in the Pasteur Education and Research Training Laboratory, Guntur.

Figure 1: Snakehead fish and Snakehead fish supplement

2.2. Nutritional Analysis in *Channa striata*

Nutritional analysis is the process of determining the nutritional content of food. It is a vital part of analytical chemistry that provides information about the chemical composition, processing, quality control and contamination of food. It ensures compliance with trade and food laws. There are a variety of certified methods used for performing nutritional analysis.

2.2.1 MOISTURE

Water is one of the most crucial components of many food products .According to authors quality, shelf life and sensory features of the product depend on the quantity of water stored in it. Therefore, the water content should be precisely determined and controlled during the product manufacturing process.

Microwave radiation method is also an extremely rapid method of drying up a sample, but the temperatures achieved are very high, making it suitable only for very thermostable materials. Larger samples can be used but the level of control of heating is reduced. Like the infrared method, the sample is typically destroyed by the analysis. It is also not useful if the moisture content is below 2%. An example of the use of the microwave method is a moisture content meter developed by the United States Department of Agriculture, which was integrated into a convection drying system for food. This allows real-time moisture determination of a food kernel without shelling the food.

Infrared radiation (IR) is used in many moisture analyzers, such as halogen moisture analyzers which are used to produce infrared radiation from a halogen lamp. IR radiation wavelength emitted by the infrared radiator is strictly conditioned by the IR radiator temperature. The weight of the sample is measured and recorded continuously and once it becomes constant the drying is stopped. The difference in the weight of the sample at the end of drying is used to calculate the moisture percentage. Halogen lamps are used in preference to ordinary

infrared generators as they are much lighter and therefore achieve maximal heat output very fast and allow excellent control of the heating process as they heat up and cool down rapidly. They also distribute the heat uniformly over the sample surface which promotes good reproducibility. The infrared radiation in such devices is absorbed by the moisture analyzer and this further reduces the time taken to heat up the sample. The infrared and halogen moisture analyzers are destructive to the sample. However, because of the speed of analysis, this technique is suited for qualitative in-process use.

2.2.2 PROTEIN

For many years, the protein content of foods has been determined on the basis of total nitrogen content, while the Kjeldahl method has been almost universally applied to determine nitrogen content. Recently, an automated instrumental technique has been developed which is capable of rapidly measuring the protein concentration of food samples. This technique is based on a method first described by a scientist called Dumas over a century and a half ago. It is beginning to compete with the Kjeldahl method as the standard method of analysis for proteins for some foodstuffs due to its rapidness

2.2.3 TOTAL FAT

The commonly used techniques are Soxhlet analysis and acid/alkaline hydrolysis . A new method based on an innovative microwave-assisted extraction (MAE) technique allows the determination of total fat in cheese samples. MAE method is statistically equivalent to the other method, showing good performance indicators (limit of quantification; LOQ = 0.248%, limit of detection; LOD = 0.087%, expanded uncertainty; U = 2.65%) and allows the determination of total fat in 12 cheese samples simultaneously in 100 min.

2.2.4 TOTAL DIETARY FIBRE (TDF)

Rapid Integrated Total Dietary Fibre (RITDF) (AOAC 2017.16) is the current technique for TDF; and closely resembles AOAC 2009.01. This method addresses the minor limitations that have been identified in the McCleary Method (AOAC 2009.01) and is the only method that accurately measures all components of TDF (including all forms of resistant starch) (Mc Cleary *et al* 2010). Several problems/challenges that became evident with the initial procedure have been resolved; enzyme levels have been optimized allowing an incubation time (4 h) consistent with human ileostomy transit time; problems associated with measurement of fructo-oligosaccharides (FOS) have been resolved by using a different high performance liquid chromatography (HPLC) system, meaningful analytical results have been obtained for phosphate-crosslinked starch.

2.2.5 ASH

Ash is inorganic residue remaining after water and organic matter have been removed by presence of oxidizing agents, which provides a measure of total minerals within a food. The most commonly used process is dry ashing. A muffle furnace is used to burn down the sample. The temperature of the chamber is maintained to approximately 600 ◦C. During this process, most of the minerals get changed into phosphates, sulphates and oxides. Due to the presence of some volatile materials in the sample, the test results are prone to being inaccurate. Therefore, other testing methods are preferred when materials like lead, mercury and iron are present in the sample. The Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) method requires a small drop/amount of sample on the ATR base-plate, being much faster than traditional techniques, allowing potential applications for simultaneous determination of sulphur, nitrogen, and ash contents for routine analysis of selected plant tannins by FTIR data.

2.2.6 TOTAL SUGAR

Sugars refer to all mono- and disaccharides present in food. Glucose, galactose, and fructose are common monosaccharides, whereas common disaccharides include lactose, maltose, and sucrose. Quantification of sugars are currently determined via several methods. This include enzymatic method that measures sucrose hydrolysis and phosphorylation of glucose and fructose or measuring absorbance increase according to standard assay of sugar kit . In addition to this, HPLC technique coupled with refractive index detector (RID) or evaporative light scattering detector (ELSD) are also used. HPLC-ELSD presents numerous advantages in terms of its sensitivity,

stability and compatibility with gradient elution, compared to HPLC-RID [Zacharis, C. K., & Tzanavaras, P. D. (2013). Dong 2006]. Recent advances in gas chromatography-mass spectrometry (GC-MS) equipment and columns have resulted in this method becoming useful in compositional and structural analysis of monosaccharides, oligomers and polymers, especially in the environmental and life sciences fields

2.2.7 MINERALS

Minerals and trace elements are naturally occurring inorganic substance that account for about 4% of total human body mass. Its serve as materials and regulators in numerous biological activities in body structure building and needed for good health. Approximately 30 elements have been recognized as essential. Minerals are grouped into two main categories: major minerals and trace minerals. Major minerals (calcium, potassium, magnesium, phosphorus, sodium, sulphur) are required in higher quantities in daily diet, while trace minerals (chromium, iron, copper, iodine manganese, molybdenum, selenium, zinc) are only needed in smaller amounts.

2.2.8 ESTIMATION OF IRON

Take 2g of sample was ashed by ignition when ashing had been completed; 5ml of HCL was added and made up to 100ml in SMF. Different concentrations of standard (Ferrous ammonium sulphate) was taken (1-5ml) corresponding to 10-50 gamma in a series of tubes. 2ml of the unknown solution was taken in test tubes. 1ml of 30% Sulphuric acid, 1ml of potassium persulphate and 1.5ml of potassium thiocyanate was added to all test tubes. This was made up to 10ml using distilled water. A blank was prepared by adding all reagents 15 expect standard and sample. All tubes were kept for 20 min incubation for color development. The intensity of the color was developed was read at 550nm in a colorimeter (AOAC 2000).

2.2.9 ESTIMATION OF VITAMIN C

Take 5g of the sample was weighed and made up to 100ml using 4% oxalic acid. 5ml of the made up sample were taken in a conical flask and titrated against 2, 6 dichloro–phenol-indophenols dye (42mg of sodium bicarbonate + 52mg of 2, 6 dichloro phenolindophenols in 50ml of water, diluted to 200ml filtered and used). The end point was appearance of pale pink color which persisted for 30 sec. The titration was repeated till concordant values were obtained. Same procedure was followed for standard. Ascorbic acid was used as standard (AOAC 2000)

2.2.10 ESTIMATION OF CALCIUM

Ash from ignited sample was dissolved in hydrochloric acid and made up to 100ml. 10ml of the ash solution was pipette out in a conical flask and 90 ml of distilled water was added to it. Add 2 drops of methyl red indicator. It was made strongly alkaline by adding ammonia and kept for boiling. 20ml of saturated ammonium oxalate was added of the solution, 10ml each time to ensure complete precipitation directly. When it was hot a few drops of acetic acid was added to render the medium acidic. The precipitate was allowed to settle overnight. The next morning the solution was filtered with Whatman No 40 filter paper. The precipitate was washed first with ammonia cal water and then with hot water several times until it was free from chloride. To test it 5ml of the washing was collected in a test tubes and a drop of silver nitrate solution was added. The washing was continued till there was no precipitate. The filter paper was collected in a flask by making a hole in the filter paper. To this 2ml of 2N sulphuric acid was added and heated at 60-80°C and when still hot was titrated against N/100 potassium permanganate solution used up the milligram of calcium present in 100g of the sample was calculated. Oxalic acid was used as the standard (AOAC 2000).

2.3 Microbial Analysis in *Channa striata*

Pour plate method (Serial dilution technique) was used for the isolation of spoilage causing bacteria and fungi from the collected Guntur Market fish. In this method, one gram of muscle was obtained from the fish and homogenised with 100 ml of distilled water and it was serially diluted upto 10-6 by following the standard procedure. Then, one ml of serially diluted samples from each concentration of samples were transferred to sterile petridishes and evenly distributed. Sterile Nutrient agar and Sabouraud's dextrose agar was poured into the sample containing petridishes and allowed to solidify. The Nutrient agar plates were incubated at 37 °C for

24 hrs and Sabouraud's dextrose agar plates were incubated at room temperature for 3 days. After incubation, the bacterial colonies were isolated from the plates and microbial population was counted by using Quebec colony counter and the enumerated colonies were expressed as cfu/ml. Well grown bacterial and fungal colonies were maintained on Nutrient agar and Sabouraud's dextrose agar slants, respectively and stored at 4^0c.

All the samples were homogenized and subjected to serial dilution.
The following is the serial dilution procedure

1. Take 10 sterile and clean test tubes.
2. The selected crushed samples [fish] were taken into a test tube and the remaining 9 test tubes were filled with 9 ml of sterile diluent such as distilled water .
3. Sterile pipette was taken.
4. 1ml of each samples were taken into the sterile pipette. The samples must be properly mixed.
5. Then transfer this 1ml of sample within the first test tube to make the total volume of 10 ml. It provides an initial dilution of 10^{-1}. Make sure during the transfer, the tip of pipette doesn't touch the wall of test tube or no amount of sample remains at the tube wall.
6. The sample was mixed properly with the diluent by shaking the tube.
7. Now discard the pipette tip and a new pipette tip was added to the pipette.
8. 1 ml of mixture sample was transferred from the 10^{-1} dilution to the second tube by using pipette. The 2^{nd} tube now has a total dilution factor of 10^{-2}
9. Repeat step 8 for the remaining tubes, 1 ml was transferred from the previous tube to the next 9 ml diluents.
10. The dilution for the bacteria/cells in the last test tube will be 10^{-9} (1 in 1,000,000 000).

Figure 2: Serial dilution procedure

2.3.1 C**ulture media preparation:-**

Sabouraud Dextrose Agar Medium

Table 1: Sabouraud Dextrose Agar Medium composition

S.NO	COMPONENTS	QUANTITY
1	Water	1000 ml
2	Peptone	10 gm
3	Dextrose	40 gm
4	agar powder	15 gm
5	final pH	5.6 +/- 0.2

- All the ingredients were combined in a 900 ml of deioinized water.

- pH was adjusted to 5.6 with hydrochloric acid and final volume was adjusted to 1 liter.
- The medium completely gets dissolved by boiling.
- Autoclaved at 121ºC for 15 minutes.
- Cool to ~45 to 50°C and poured into petri dishes or tubes for slants.

Figure 3: Sabouraud Dextrose Agar Medium

2.3.2. Nutrient Agar Medium

Table 2: Composition of Nutrient Agar Medium

S.NO	COMPONENTS	QUANTITY
1	Water	1000 ml
2	Peptone	5 gm
3	Beef extract	2 gm
4	NaCl	5 gm
5	Agar powder	15 gm
6	Final pH	7.2 +/- 0.2

- All the ingredients were combined in a 900 ml of deioinized water.
- pH was adjusted to 7.2 with hydrochloric acid and final volume was adjusted to 1 liter.
- The medium completely gets dissolved by boiling.
- Autoclaved at 121ºC for 15 minutes.
- Cool to ~45 to 50°C and poured into petri dishes or tubes for slants.

Figure 4: Nutrient Agar Medium

3.0 RESULTS AND DISCUSSION

The results of the proximate composition were presented. Moisture content was highest in proximate composition, followed by carbohydrates and protein. Ash value was low compared to other contents. The Fat, Total dietary fiber, ash, total sugars, minerals, iron, vitamin C and calcium content were analysed.

Table 3: Nutritional parameters of *Channa striata*

Nutritional Parameters	***Channa striata***
Moisture	50.05%/g
Protein	6.04%/g
Fat	1.02%/g
Total dietary Fiber	1.08%/g
Ash	0.01%/g
Total Sugar	11.03%/g
Minerals	45.01%/g
Iron	0.82mg/g
Vitamin-C	0/g
Calcium	5.38mg/g

3.1 Microbiological analysis

The microbiological characteristics of snakehead fish flour and its modifications. The microbiology characteristics showed that the total plate count was 2.2 x 10^5 colony/g, the total of yeast and mold was 1.5 x 10^2 colony/g, and the total amount of Staphylococcus aureus was <1.0 x 101 colony/g.

Table 5: Microbiological data analysis of *Channa striata*

Type of analysis	**Unit**	**Results**
Total plate count	Colony/g	2.4×10^5
Total of yeast and mold	Colony/g	1.7×10^2
Salmonella	-	Negative
Escherichia coli	MPN/g	Negative
Total of Staphylococcus aureus	Colony/g	<1.0 x 101
Coliform	MPN/g	0

Figure 5: Microbiological characteristics of *Channa striata*

4.0 Conclusions

The original snakehead fish and modified snakehead fish flour (snakehead fish flour with tofu dregs and snakehead fish flour with turmeric extract) have met the quality requirements for a fish meal based on SNI 01-2715-1996 (improvement of SNI 01-2715- 1992 revision). In general, the three types of snakehead fish flour in this study meet the Grade I criteria (nutritional and microbiological characteristics). So that, the three snakehead fish flour can recommend being used as a weight enhancing supplement for children with tuberculosis. But, several stages include organoleptic and experimental research, need to be done to prove the effectiveness of these supplements on weight gain in children with tuberculosis. Among the three types of snakehead fish flour, the original snakehead flour meets the criteria closest to Grade I. However, better processing technology for snakehead fish supplements is needed to improve the quantity and quality of nutritional content, especially protein and albumin content and microbiological characteristics. Furthermore, prevention and control are necessary for every stage in making snakehead fish supplements using the Hazard Analysis Critical Control Point (HACCP) for food quality assurance.

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