ISBN: 978-81-19149-98-8

3. Analytical Methods of Proximate Composition of Food Fishes

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Abstract:

Fish are the cool-blooded animals found in three different habitats including freshwater, saltwater or both. These habitats are different based on several factors including temperature, light, pH, dissolved gases and salts in water, turbidity, alkalinity, and depth. In fact, because the fish live in different geographical areas, any differences in ecosystem such pH, temperature, water quality can also affect their nutritional contents (Tilami et. al 2018). Fish are good sources of high-quality protein, with amino acid composition which very well suited to human dietary requirements comparing favorably with egg, milk and meat.

It contains unsaturated fatty acids and other nutrients including vitamins and minerals that are beneficial to the human body (Ugoala, et al., 2009, Nurnadia, et al., 2011, Babji, et al., 2015, Priatni et.al 2018). Freshwater fish also contain beneficial polyunsaturated such as eicosapentaenoic acid (EPA) and docasahaexaenoic acid (DHA), although these were less common than that found in marine fish. Fish is rich in macronutrients and micronutrients such as protein, carbohydrate, fat and amino acids. Proximate analysis is vital in the food industry for the development and quality controls of food products. Knowledge and information regarding the proximate composition of fish are essential in the food processing technology of fish products at commercial and industrial scales.

Keywords:

Fish, Proximate composition, Protein, fatty acids, nutrients

3.1 Introduction:

The chemical composition of fish varies greatly from one species and one individual to another depending on sex, age, environment and season. Therefore, a substantial normal variation is observed for the constituents of fish muscle.

Proximate analysis is defined as the determination of a group of closely related components together. It includes determination of amount of moisture, protein, fat (ether extract), ash and fibre. Fish muscle comprises of moisture, protein and fat as a major nutrient components and carbohydrates, vitamins and minerals as minor components. Fish muscle contains all the nutrient components that is required most for human body maintenance. Fish and fish products are the most important sources of animal protein in the human diet. It comprises of all the ten essential amino acids in desirable quantity for human consumption.

Fish protein is very rich in such amino acid as methionine, lysine and low in tryptophan compared to mammalian protein (Nowsad, 2007).

Fish have rich source of essential nutrients required for supplementing both infant and adult diets (Abdullahi et al., 2001). Fish normally has more poly unsaturated fatty acids than animal fats. Since their importance from medical point of view is obvious. An increasing amount of evidences suggest that due to its high content of polyunsaturated fatty acid fish flesh and fish oil are beneficial in reducing the serum cholesterol (Stansby, 1985). The molecular and elementary composition gives the content of the different compounds that are of commercial value. (e.g. moisture content, ash content, crude proteins, crude fat) and also help us in understanding the importance of fish as a food item

3.2 Estimation of Moisture Content:

The water content of fish may vary between 28% and 90%. In each species of fish, the water content is highly variable due to growth, maturity, spawning, feeding and starvation.

However, water content has an important role in deciding the shelf life and texture of a food material. The higher the water content, the higher is the chance of spoilage and vice versa.

In food analysis, sample is made moisture free to collect the dry matter in a food. Estimation of moisture can be done by method suggested by AOAC, (2000).

It is prerequisite to conduct moisture analysis in order to determine other constituents in food. Food stuffs contain moisture in three forms as:

- Free water that is moisture in the inter granular space of food material. Example: fruits.
- Absorbed water that is water under the surface of macromolecules. Example: soaked pulses.
- Bound water that is water in combination with other substances. Example: water found as a constituent of carbohydrates, fats in food material.

A. Principle:

Food material is heated under specific conditions, usually by thermal drying. Loss in weight occurs during heating due to volatilization of water vapor from food.

This loss in weight is taken as a measure of moisture content of the sample. Theoretically weight loss should result from loss of water, from food but in practice, volatilization of organic components also takes place.

B. Procedure:

Weigh 5 to10 g sample (A) in a clean, dry, weighed petri plate or porcelain dish (B). Cover it with aluminum foil. Keep it in oven at 50-70oc, sweet products required to be dried at 30 to 500c to prevent charring of sugar. Cool in desiccator and weigh again (C). Repeat the procedure till constant weights are obtained.

C. Calculation:

$$* \textbf{Moisture } \left(\frac{\text{g}}{100\text{g}}\right) = = \frac{\text{Initial reading (B)} - \text{ Final reading (D)}}{\text{Weight of sample taken (A)}} \text{x } 100$$

Reading: AOAC (2000).

3.3 Estimation of Crude Protein:

Micro Kjeldahl method is commonly used to determine the protein content of foodstuffs by estimating the nitrogen content of the material. A food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food and multiplying nitrogen value by 6.25 (general factor). It is considered as crude protein because non protein nitrogen (NPN) present in food as purine, pyrimidine base, vitamin, amino sugar, alkaloids, compound lipid etc. is also included in total nitrogen (AOAC, 1990).

A. Principle:

The nitrogen presents in protein or any other organic material is converted to ammonium sulphate by sulfuric acid (an oxidizing agent which digests the food), during digestion.

Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia, and other organic matter to C02 and H20.

Ammonia gas is not liberated in an acid solution as it is in the form of the ammonium ion (NH_4^+) which binds to the sulfate ion (SO_4^{2-}) and thus remains in solution. This solution is made alkaline with the addition of sodium hydroxide solution. This salt, on steam distillation liberates ammonia which is collected in boric acid solution. Ammonia forms a loose compound, ammonium borate with boric acid and titrated against standard acid.

B. Materials Required:

Equipment/apparatus: Micro kjeldahl flasks weighing balance, kjeldahl digestion unit, nitrogen distillation unit.

Glass wares: Tubes, pipettes, measuring cylinder, conical flask, burette, volumetric flask.

C. Reagents:

a. Conc. Sulphuric acid (A.R. grade)
b. Sodium hydroxide (40%): Dissolve 200 g of NaOH in little water and make volume to 500 ml.
c. Boric acid (4%): Dissolve 4 g of boric acid in 100 ml distilled water.
d. Hydrochloric acid (0.02 N): Dilute 0.85 ml HCl to 500 ml with distilled water. Test the normality of HCl with 0.02 N NaOH which has already been standardized with 0.02 N oxalic acid.
e. Digestion mixture: Mix 98 parts of potassium sulphate and 2 parts of copper sulphate uniformly.
f. Indicator: (a) Methyl red (0.2%): Dissolve 200 mg of methyl red in 100 ml alcohol. (b) Methyl blue (0.2%): Dissolve 200 mg of methyl blue in 100 ml alcohol. (c) Mix 2 parts of 2% alcoholic methyl red solution with 1 part of 0.2% alcoholic methyl blue.

D. Procedure:

The Kjeldahl method can conveniently be divided into three steps: digestion, neutralization and titration.

I Step Digestion:

Weigh 100 mg of moisture free powdered sample in triplicate. Put it into micro Kjeldahl flasks.

Add 0.5 g of digestion mixture and 2 ml of conc. H2SO4 (AR grade), keep one sample blank i.e. without sample. Heat the tube for 10-12 hrs on digestion rack till the contents are digested and clear solution is obtained. Cool the sample.

II Step Distillation:

Boil water in the distillation flask. Allow the steam to pass through the condenser. Rinse the apparatus 2-3 times by passing the steam. Transfer the digested mixture into the distillation flask. Rinse the digestion tube 3-4 times with distilled water.

Add 10 ml of NaOH to it. Transfer 5 ml of boric acid and few drops of indicator in a conical flask. Place the flask under condenser keeping the tip of the condenser dipped/ touched with the surface of solution to prevent loss of ammonia.

Collect the ammonia liberated in the flask, the color will turn to light green. Remove the flask when volume of distillate is about 15 ml.

III Step Titration:

a. Put standardized 0.02 N HCl in a burette. Dilute the distilled content with 50 ml water. Titrate till gray/pink color is obtained. Record the amount of acid consumed.

E. Calculation:

1 ml of 0.02 N HCl 14.009g nitrogen

$$* \textbf{Percent nitrogen} = = \frac{(\text{ml HCl in determination} - \text{ml blank}) \text{ x Normality of HClx14 x100}}{\text{Weight of the sample (mg)}}$$

The protein content of food is calculated by multiplying the nitrogen value with the general factor 6.25 assuming that average nitrogen content of most of the protein is 16% therefore 1 g nitrogen is equal to 6.25 g protein (100/16).

Reading: AOAC (2000).

3.4 Estimation of Crude FAT:

Fats and fatty acids are the esters of glycerol. Fat is estimated as crude ether extract of the dry material which include true fats, fatty acids, sterols, chlorophyll and other pigments like phospholipids, carotenoids etc. (AOAC, 2000).

A. Principle:

Fat is estimated as crude ether extract of the dry material which includes true fats, fatty acids, sterols, chlorophyll and other pigments like phospholipids, carotenoids etc.

Oil from food is solubilized in petroleum ether and then distilled off completely to estimate the crude fat in the sample.

B. Materials Required:

Equipment's/Apparatus: Weighing balance, hot plate, Soxhlet apparatus

Glass wares: Flat bottom flask

Others: Absorbant cotton, whatman no. 2 filter paper

Chemicals: Petroleum ether (40-60oc)

C. Procedure:

Weigh 5-10 g of moisture free sample –(A) (amount of sample vary with fat content of the sample). Fold a piece of whatman filter paper in such a way to hold the food sample. Wrap around another filter paper which is left open at the top like a thimble.

Place a piece of cotton at the top to evenly distribute the solvent as it drops on the sample during extraction. Place the sample packet in the extracting tube (with siphon arm) of soxhlet apparatus.

Fill flat bottom flask with 200 ml of petroleum ether. Attach flat bottom flask to extracting tube then connect to condenser. Run the apparatus for 16 hours to ensure complete extraction of fat by gentle heating.

Check for continuous water flow in the condenser during extraction. Allow to cool and dismantle the flask. Evaporate the ether on steam or water bath until no odor of ether remains. Cool to room temperature and weigh(B).

D. Calculation:

$$* \textbf{Fat}\ (\mathrm{g}/100\mathrm{g}) = = \frac{\text{Wt. of ether extracted fat } (\mathrm{B}-\mathrm{A})}{\text{Wt. of sample } (\mathrm{g})} \mathrm{x}\ 100$$

Reading: AOAC 2000.

3.5 Estimation of ASH:

Ash is the inorganic residue remains after burning of organic matter. Ash of fruits and vegetables is alkaline in nature while meat and cereals have acidic ash.

Alkaline ash is due to the presence of salts or organic acids that are converted to carbonates during ashing. Estimation of total ash is an index of refinement of foods and a useful parameter of nutritional value of foods.

A. Materials Required:

Equipment's	:	Muffle furnace, clay pipe triangle, hot plate, Weighing balance and desiccator.
Glass wares	:	Crucibles

B. Procedure:

Weigh about 5-10 g of the sample in a porcelain or crucible, prior to this it is suggested to heat crucible to about 550^o10^oc and cooled to avoid error. Place the crucible on a clay pipe triangle or hot plate.

Put the material over low flame till the sample is charged. Keep the charged sample in a muffle furnace for about 3-5 h at about 600^oc. Switch off the furnace and allow to cool. Cool the sample in a desiccator and weigh.

Heat the crucible again in a muffle furnace for 1 hour. Cool in desiccator and weigh. Repeat the process till two consecutive weights are similar and the ash is almost white or grayish in color.

C. Calculations:

$$* \text{Ash (g/100g)} = = \frac{\text{Weight of the ash (g)}}{\text{Weight of sample taken (g)}} \text{x } 100$$

Reading: AOAC (2000).

3.6 Estimation of Crude Fibre:

Crude fibre is a loss on ignition of dried residue remaining after digestion of sample with 1.25% Sulphuric acid and 1.25% sodium hydroxide solutions under specific conditions.

A. Principle:

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur.

The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fibre content.

B. Material Required:

Equipment's/Apparatus: Weighing balance, muffle furnace, desiccator, hot plate, spatula

Glass wares: Graduated beakers, conical flasks, funnel, crucibles

Others: Whatman paper 54

C. Reagents:

a. Sulphuric acid (0.255 N/1.25%): Add 7.2 ml of Sulphuric acid to 500 ml of distilled water and make volume to 1 litre
b. Sodium hydroxide (0.313 N/1.25%): Dissolve 13.16 g of sodium hydroxide in little amount of water and make volume to 1 litre.
c. Alcohol
d. Ether

D. Procedure:

Make the sample moisture and fat free. Sample can be made fat free by dipping in fat solvent overnight or by following the methods explained above. Add 200 ml of 1.25% Sulphuric acid to beaker.

Boil the mixture for 30 min keeping the volume constant by the addition of water at frequent intervals. Acid will hydrolyze the protein and carbohydrates present in sample.

Filter the mixture through whatman paper 54. Wash the residue with hot water till free from acid. Transfer the residue to the beaker again. Add 200 ml of 1.25 percent NaOH.

Boil the mixture for 30 minutes by keeping volume constant with distilled water. Sodium hydroxide will saponify the fatty material if present in sample. Filter the mixture through whatman paper 54.

Wash the residue with hot water till free from alkali. Wash with some alcohol and ether. Transfer the residue to a crucible which has been dried overnight at 80-100^{o}c and weighed (w_1).

Dry the residue in oven at 130^{0}C for 2 to 3 hrs. Cool the crucible in desiccator and weigh (w_2). Ignite in a muffle furnace at 600^{o}c for 2 to 3 hours, till it is converted into ash, cool and weigh again (W_3).

E. Calculation:

$$\text{Percent Crude fibre} = \frac{(w_2 - w_1) - (w_3 - w_1)}{\text{wt of sample}} \times (100)$$

Reading: AOAC (2000).

3.7 Estimation of Carbohydrate:

Carbohydrate content of the sample can be determined by difference method, i.e. sum of moisture, protein, crude fibre, fat, and ash, subtracted from 100.

A. Calculation:

Carbohydrate content (g/100g) = [100 – (moisture + protein + crude fibre + fat + ash)]

3.8 Estimation of Energy Content:

Energy content of the food sample is determined by using the physiological fuel value of protein, fat and carbohydrate are 4, 9 and 4 kcal per gram respectively.

Using factor of 4, 9 and 4, the energy content of food sample can be derived by using following formula.

A. Calculations:

Energy content (kcal/100g) = (% protein x 4) + (% carbohydrate x 4) + (% fat x 9)

3.9 References:

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