ANALYTICAL METHODS

1. Determination of moisture content (AOAC, 2000)

Method

1. Dry the empty dish and lid in the oven at 105°C for 3 h and transfer to desiccator to cool. Weigh the empty dish and lid.
2. Weigh about 3 g of sample to the dish. Spread the sample to the uniformity.
3. Place the dish with sample in the oven. Dry for 3 h at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample.

Calculation

$$\text{Moisture (\%)} = \frac{(W1-W2)}{W1} \times 100$$

where: W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying

2. Determination of protein content (AOAC, 2000)

Reagents

- Kjedahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) with 1 part of coppersulphate ($CuSO_4$)
- Sulfuric acid (H_2SO_4)
- 40% NaOH solution
- 0.2 N HCl solution
- 4% H_3BO_3
- Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5-1.0 g) in digestion flask.
2. Add 5 g Kjedahl catalyst and 200 ml of conc. H_2SO_4
3. Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently unit frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml of distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 drops of mix indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.
6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\text{Protein (\%)} = \frac{(A-B) \times N \times 1.4007 \times 6.25}{W}$$

Where A = volume (ml) of 0.2 N HCl used sample titration

B = volume (ml) of 0.2 N HCl used in blank titration

N = Normality of HCl

W = weight (g) of sample

14.007 = atomic weight of nitrogen

6.25 = the protein-nitrogen conversation factor for fish and its by-products

3. Determination of ash content (AOAC, 2000)

Method

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned off.
2. Cool the crucible in the desiccator (30 min).
3. Weigh the crucible and lid to 3 decimal places.

4. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.
5. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
6. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for the further ashing.

Calculation

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

4. Determination of fat content (AOAC, 2000)

Reagents

- Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105°C overnight to ensure that weight of bottle is stable.
2. Weigh about 3-5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into soxhlet.
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/min).
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the bottle at 80-90°C until solvent is completely evaporate and bottle is completely dry.
9. After drying, transfer the bottle with partially covered lid to the desiccator to cool. Reweigh the bottle and its dried content.

Calculation

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

5. Determination of lipid content (Bligh and Dyer, 1959)

Reagents

- Chloroform
- Methanol

Method

1. Homogenize the sample (20 g) with 16 ml of distilled water, 40 ml of chloroform and 80 ml of methanol at the speed of 9,500 rpm for 1 min at 4°C.
2. Add 40 ml of chloroform and homogenize for 30 sec.
3. Add 40 ml of distilled water and homogenize again for 30 sec.
4. After centrifugation of the homogenate at 2,000 rpm at 4°C for 20 min, transfer the supernatant into a separatory funnel and allow to separate.
5. Determine lipid content gravimetrically by measuring triplicate aliquots of the chloroform layer into tared containers, evaporate the solvent and weigh.
6. Calculate the lipid content.