16. Quality Evaluation of fish meal

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Analysis of Proximate Composition of fish and fish meal

Proximate composition is the composition of major nutrients. In case of fish meal, it is the composition of moisture, protein, fat and ash. Variation in the values of different components of fish meal are observed due to variation in fish species used for production of fish meal, season of fishing the raw material, etc.

Sample Preparation

In order to evaluate proximate composition of raw material for fish meal, sample preparation is an essential step. For fish meal samples, direct sampling of fish meal can be carried out in order to evaluate proximate composition.

For raw material used

Cut the fish/ raw material into small pieces after removing the fins, skin, bones etc. and mix well. Grind the pieces as smoothly as possible for obtaining a homogeneous sample using a mixer grinder. Keep the material in an air-tight container, at low temperature to prevent the loss of moisture during subsequent handling. Use this material for further analysis.

A. Estimation of Moisture Content in Fish and Fish Meal

Principle

Determination of moisture is made by drying the sample at elevated temperature. Percentage of moisture is derived from the difference in weight of the sample before and after drying.

Apparatus

1. Analytical Balance
2. Drying Hot air oven (at 100 + 2 ^{0}C)
3. Glass dishes with cover (Petri dishes)
4. Desiccators containing absorbing element ($CaCl_2$)

Procedure

1. Weigh approximately 10 g of the prepared sample. Use spatula to weigh the sample in a previously weighed glass dish with cover (petri plates with lids).
2. Dry in a hot air oven at 100 ^{0}C to a constant weight for 16-18 hrs.
3. After drying time is over, remove sample from the oven, cover the petri dish and place in desiccating unit.
4. Cool to room temperature for about 30 minutes and weigh the plates with lid accurately.

Calculation

$$\boldsymbol{Mositure\ Content}\ (\%) = \frac{(\boldsymbol{Initial\ weight\ of\ petriplate+sample-Final\ weight\ of\ petriplate+sample\ after\ drying})}{(\boldsymbol{Initial\ weight\ of\ crucible+sample-Weight\ of\ empty\ crucible})}\ \boldsymbol{X\ 100}$$

Loss in weight can be calculated from difference in Initial weight of empty petri plate with lid before drying + sample and final weight of petri plate with lid + sample after drying

B. Estimation of crude protein content in fish and fish meal

Principle

The Kjeldahl method for determining total nitrogen involves, first heating with concentrated sulphuric acid in a long-necked digestion flask. The reaction rate is accelerated by adding Sodium or potassium sulphate to raise the boiling point from 324 ^{0}C to 400 ^{0}C. Carbon and hydrogen of the organic matter are oxidized to CO_2 and H_2O. A part of H_2SO_4 is simultaneously reduced to SO_2 which in turn reduces nitrogenous material to NH_3. The NH_3 combines with H_2SO_4 and remains as $(NH_4)_2SO_4$. After making alkaline with concentrated NaOH solution, the ammonia is distilled and absorbed in boric acid as ammonium borate. Ammonia is titrated in the standard acid.

Reagents

1. Concentrated H_2SO_4 (93-98%) N_2 free
2. Digestive Mixture [96% anhydrous sodium (Pot.) Sulphate + 3.5% Copper sulphate ($CuSO_4.5H_2O$) + 0.5 % selenium dioxide (SeO_2) -act as catalyst]
3. Mixed indicator (0.016% methyl red & 0.083% Bromocresol green in alcohol)
4. Stones or glass beads to prevent bumps while heating.
5. 40% NaOH Solution
6. 0.1 N H_2SO_4
7. Phenolphthalein indicator
8. 2% Boric acid

Apparatus

1. Kjeldahl digestion flasks and distillation apparatus complete with heat source, bulbs & delivery tubes
2. Distillate receiving flasks (150 ml conical flasks)
3. Burettes (50±0.1 ml volume)
4. Bulb pipettes (5 ml and 10 ml volume)
5. Standard Volumetric flask (50 ml/ 100 ml volume)

Procedure

1. Weigh accurately 1 to 2 g of the prepared sample or 0.5 g of fish meal sample and transfer into Kjeldahl digestion flask of 100 ml capacity.
2. Add a few glass beads and pinch of digestion mixture and 10 ml of concentrated AR sulphuric acid.
3. Digest over electrical heater till solution turns colorless.
4. Cool and dilute this digested solution up to 50 ml with double distilled water.
5. In receiving flask, take 10 ml of 2% Boric acid with mixed indicator.
6. To the Kjeldahl distillation flask, add 5 ml of diluted and digested solution and rinse down with distilled water.
7. Add two drops of phenolphthalein indicator and 40% NaOH (10 ml) till the indicator changes to pink.
8. Connect the Kjeldahl flask to the bulb of distillation rack and apply heat in order to collect at least 30-40 ml of distillate (distill for 4 minutes)
9. Determine the amount of absorb ammonia in 2% boric acid, by titrating with 0.1 N H_2SO_4.

Calculation

1 ml. of 0.1 N H_2SO_4 = 0.0014 g of Nitrogen

$$\textbf{\textit{Crude Protein}}\ (\%) = \frac{\mathbf{0.0014}\ \boldsymbol{X}\ \boldsymbol{TV}}{\textbf{\textit{Weight of sample taken for digestion}}}\ \boldsymbol{X}\ \frac{\mathbf{50}}{\mathbf{5}}\ \boldsymbol{X}\ \mathbf{100}\ \boldsymbol{X}\ \mathbf{6.25}$$

Where,

6.25 is empirical factor for fish protein determination from nitrogen

TV is the titre value of 0.1 N H_2SO_4

C. Estimation of crude fat content in fish and fish meal

Principle

Fat content of moisture free sample is determined by extracting the fat with a suitable solvent (diethyl ether or petroleum ether (Boiling Point 40 to 60 ^{0}C) using Soxhlet apparatus. The solvent is removed from the extract by evaporation and residue is weighed and reported as fat.

Reagent

1. Petroleum ether (Boiling Point 40-60 ^{0}C)

Apparatus

1. Soxhlet Extraction Apparatus complete
2. Thimbles
3. Hot air oven (100 ^{0}C)
4. Desiccator
5. Electric Heater

Procedure

1. Weigh a clean dry Soxhlet extraction flask.
2. Place a weighed moisture free well ground (powdered) dried sample (2-3 g) into a Soxhlet extraction thimble.
3. Place extraction thimble in the extractor with an attached receiving flask and pour the solvent, washing into the thimble through glass funnel.
4. Connect the extractor & receiving flask to the Soxhlet condenser.
5. Adjust the electrical heating unit so that the solvent syphons over 5 to 6 times per hour and extract the fat on the Soxhlet apparatus for about 12 hrs.

6. Remove the extraction thimble & place in hot air oven for drying.

7. After extraction, evaporate the petroleum ether in the extraction flask over a hot water bath.

8. When no odour of ether remains, dry the flask in a hot air oven (100±2 ^{0}C) for 1-2 hrs.

9. Cool in desiccator for 30 minutes and weigh.

10. Determine weight of the fat.

Calculation

$$\boldsymbol{Crude\ Fat\ content}\ (\%\ \boldsymbol{on\ dry\ weight\ basis}) = \frac{\boldsymbol{Final\ weight\ of\ flask + fat - Weight\ of\ empty\ flask}}{\boldsymbol{Weight\ of\ sample}}\ \boldsymbol{X\ 100}$$

In order to convert fat into original weight basis (OWB)

$$\boldsymbol{Crude\ Fat\ Content}\ (\%\ \boldsymbol{on\ original\ weight\ basis}) = \frac{(\boldsymbol{100 - Mositure}\ (\%))\ \boldsymbol{X\ Crude\ fat}\ (\%\ \boldsymbol{on\ dry\ weight\ basis})}{\boldsymbol{100}}$$

This will give crude fat content in fish meal sample on original weight basis.

D. Analysis of total ash content in fish and fish meal

Principle

The objective of determining the ash in fish is to have an idea of the mineral content of fish. The general method for determining the total ash involves gently igniting the contents first and then at 500 ^{0}C in muffle furnace until the white ash is obtained.

Apparatus

1. Muffle furnace
2. Analytical balance
3. Ignited & cooled silica dish (ignite it for about an hour)
4. Tongs
5. Desiccator

Procedure

1. Weigh accurately 2-3 g of well ground (powdered), dried sample or 5 g of minced fish sample, in a previously heated, cooled & weighed silica dish (crucible).
2. Then the dish and contents are ignited, first gently & then at 500±5 ^{0}C in muffle furnace until whitish grey ash is obtained.
3. Cool in a desiccator and weigh.
4. If the starting material is wet & fresh, the dish with sample is first heated over a burner, to smoke off excess moisture, fat, etc. without burning and then transfer to the muffle furnace and heating is continued as above.

Calculation

$$\boldsymbol{Total\ Ash}\ (\%) = \frac{(\boldsymbol{Final\ weight\ of\ crucible\ with\ ash - Weight\ of\ empty\ crucible})}{(\boldsymbol{Initial\ weight\ of\ crucible + sample - Weight\ of\ empty\ crucible})}\ \boldsymbol{X\ 100}$$

Biochemical Quality Indices

Fish undergoes spoilage after death. As a result of progressive spoilage, various biochemical quality parameters change indicating spoilage stages, quality of fat, fat oxidation, degree of spoilage, etc. Various biochemical quality parameters for fish meal include analysis of total volatile base nitrogen (TVB-N), tri methyl amine nitrogen (TMA-N), peroxide value (PV), free fatty acid value (FFA), non-protein nitrogen content (NPN), etc.

E. Estimation of pH

pH is the concentration of hydrogen (H^+) ions in the sample. It is the measurement of acidity or alkalinity of the sample. pH is one of the important biochemical quality indices of fish and fish meal. Value of pH equal to 7 is considered as neutral whereas pH values less than 7 and more than 7 are considered as acidic and basic pH values respectively. It can be detected with the help of pH meter. pH meter is portable battery operated instrument with glass electrode. pH is measured by direct contact between sensitive diaphragm of electrode and sample. Difference in the electric load between sample and electrolyte solution (KCl) are measured to give pH values. Calibration of pH meter before use is necessary.

Apparatus

1. Digital pH meter
2. Distilled water
3. Electrolyte solution
4. Beaker

Procedure

1. Take 5 g of fish/ fish meal sample and homogenize with 45 ml distilled water.
2. Measure the pH by dipping the electric rod into the solution and note down. Repeat thrice and take an average value for pH reading.

F. Estimation of Total Volatile Base Nitrogen (TVB-N) in fish and fish meal samples

As fish undergoes spoilage the content of TVB-N increases. TVB-N is used to determine early stages of spoilage.

Principle

Volatile basic nitrogen content is mainly constituted by Ammonia. When tri-chloro acetic acid (TCA) extract of the sample is treated with saturated sodium carbonate, ammonia will liberated which is then trapped in N/100 (0.01 N) H_2SO_4 in the Conway dish. The excess acid in inner chamber is back titrated with N/100 (0.01 N) NaOH. The calculated value gives the TVB-N content of the sample.

Reagents

1. N/100 H_2SO_4
2. N/100 NaOH
3. Saturated sodium carbonate solution
4. Mixed indicator

Apparatus

1. Conway micro-diffusion unit
2. Graduated pipettes (1 ml volume)
3. Micropipette (1 ml volume)
4. Petroleum jelly/ Vacuum grease

Procedure

Preparation of TCA extract

In order to prepare TCA extract, 10 g of fish/ fish meal can be weighed into

mortar and 10% TCA is added to it (approximately volume is 10 ml) and the mixture is ground well with the help of pestle. The resultant solution is filtered using Whatman Filter Paper No. 1 in 50 ml volumetric flask. The extraction is repeated and solution is filtered with 10% TCA. Washings are collected and volume is made to 50 ml. The resultant filtrate after extraction with TCA is regarded as TCA extract.

Procedure

1. TVBN is estimated by the micro diffusion Conway method.
2. In the inner chamber of the Conway unit place 1 ml. of N/100 H_2SO_4 and in the outer chamber, 1ml of TCA extract of the sample.
3. Cover the Conway dish with the glass cover smeared with petroleum jelly to give air-tight contact along the outer contact-ring of the unit.
4. Keep just open to draw 1ml of saturated Na_2CO_3 in the outer chamber of the unit, then closed the glass plate to air tight.
5. Mix gently by lateral circular movement.
6. Allow the unit undisturbed to stand overnight (or at 37^0C incubation for 2 hrs.).
7. The acid in the inner chamber is titrated against N/100 NaOH using two drops of mixed indicator, the indicator changing from red to blue at the end point (B).
8. Titrate a reagent blank also by taking standard acid at the central compartment (A).
9. Perform the assay in duplicate for each sample.

Calculation

Difference in the values of A and B is the volume of N/100 Acid used up by volatile base.

1 ml of N/100 acid = 0.14 mg of Nitrogen

$$\boldsymbol{TVB - N\ (mg\ \%)}$$

$$= \frac{(\boldsymbol{Titre\ value\ of\ blank - Titre\ value\ of\ sample})\ \boldsymbol{X\ 0.14}}{\boldsymbol{Weight\ of\ sample\ taken\ for\ TCA\ extract\ preparation}}\ \boldsymbol{X}\ \frac{\mathbf{50}}{\mathbf{1}}\ \boldsymbol{X\ 100}$$

For blank, TCA is used instead of sample.

G. Estimation of Tri-methyl Amine (TMA) in fish and fish meal samples

Principle

Tri-methyl amine (TMA) is a non-protein nitrogenous volatile compound. The quantity of TMA formed is depends primarily upon the concentration of its precursor, TMA-O in the fish muscle. TMA-O is reduced during spoilage to TMA. The TMA is often determined by the Conway micro-diffusion technique.

Reagents

1. N/100 H_2SO_4
2. N/100 NaOH
3. Saturated sodium carbonate solution
4. Mixed indicator
5. Neutralized formalin (formaldehyde)

Apparatus

1. Conway micro-diffusion unit
2. Graduated pipettes (1 ml volume)
3. Micropipette (1 ml volume)
4. Petroleum jelly/ Vacuum grease

Procedure

Preparation of TCA extract

In order to prepare TCA extract, 10 g of fish/ fish meal can be weighed into mortar and 10% TCA is added to it (approximately volume is 10 ml) and the mixture is ground well with the help of pestle. The resultant solution is filtered using Whatman Filter Paper No. 1 in 50 ml volumetric flask. The

extraction is repeated and solution is filtered with 10% TCA. Washings are collected and volume is made to 50 ml. The resultant filtrate after extraction with TCA is regarded as TCA extract.

Procedure

1. TMA is also estimated by the micro diffusion Conway method.
2. In the inner chamber of the Conway unit place 1 ml. of N/100 H_2SO_4 and in the outer chamber, 1ml of TCA extract of the sample.
3. Cover the Conway dish with the glass cover smeared with petroleum jelly to give air-tight contact along the outer contact-ring of the unit. Keep it just open in order to add few more chemicals.
4. Add 0.5 ml neutralized formalin (prepared by shaking formaldehyde with magnesium carbonate and filtering through Whatman 40 no. filter paper) to the outer chamber and swirl to mix before adding saturated sodium carbonate solution. Formaldehyde is added to fix all the bases except TMA.
5. Keep just open to draw 1ml of saturated Na_2CO_3 in the outer chamber of the unit, close the glass plate to air tight.
6. Mix gently by lateral circular movement.
7. Allow the unit undisturbed to stand overnight (or at 37^0C incubation for 2 hrs.).
8. The acid in the inner chamber is titrated against N/100 NaOH using two drops of mixed indicator, the indicator changing from red to blue at the end point (B).

9. Titrate a reagent blank also by taking standard acid at the central compartment (A).
10. Perform the assay in duplicate for each sample.

Calculation

Difference in the values of A and B is the volume of N/100 Acid used up by volatile base.

1 ml of N/100 acid = 0.14 mg of Nitrogen

$$\boldsymbol{TMA}\ (\boldsymbol{mg}\%) = \frac{(\boldsymbol{Titre\ value\ of\ blank - Titre\ value\ of\ sample})\ \boldsymbol{X}\ \mathbf{0.14}}{\boldsymbol{Weight\ of\ sample\ taken\ for\ TCA\ extract\ preparation}}\ \boldsymbol{X}\ \frac{\mathbf{50}}{\mathbf{1}}\ \boldsymbol{X}\ \mathbf{100}$$

For blank, TCA is used instead of sample.

H. Analysis of Peroxide Value (PV) of fish/ fish meal sample

Principle

During oxidation of fat, peroxide is formed. Peroxide value gives a measure of oxidative rancidity. The peroxide value is a measure of peroxides contained in the oil. The peroxide value is usually determined volumetrically by method which depends on the reaction of potassium iodide in acid solution with the peroxide oxygen followed by titration of the liberated iodine with Sodium thio-sulphate solution.

Reagents

1. Glacial acetic acid.
2. 1 % starch solution (freshly prepared)
3. N/100 Sodium Thio-sulphate solution
4. Potassium Iodide

Procedure

1. In a 250 ml iodine flask, take 20 ml of chloroform extract (prepared in FFA).
2. Add about 30 ml of glacial acetic acid and 1 g of KI. Swirl and keep in dark for about 30 min. with occasionally swirling.
3. Take out and add 1 ml of 1% starch solution.
4. Titrate liberated iodine with N/100 Sodium Thio-sulphate solution.

Calculation

$$\boldsymbol{Peroxide\ Value}\ (\%) = \frac{(\boldsymbol{TV\ of\ Sample - TV\ of\ Blank})\ \boldsymbol{X\ Normality\ of\ Sodium\ thiosulphate}}{\boldsymbol{Weight\ of\ fat}}\ \boldsymbol{X\ 100}$$

Where;

TV = Titre value

I. Estimation of free fatty acid content of fish and fish meal samples

The deterioration of lipids has always been of primary concern to fishery technologists. Degradation of lipids falls into two categories: oxidation which leads to off odors and flavours and hydrolysis which splits off free fatty acids. FFA gives a measure of hydrolytic rancidity.

Principle

Fat spoilage can be assessed by estimating the free fatty acids (FFA) and peroxide value (PV) on a common chloroform extract. The FFA in the sample extract is diluted with alcohol and neutralized by titration with sodium hydroxide. The FFA content is expressed as % Oleic acid on the extracted fat.

Reagents

1. Chloroform
2. Anhydrous Sodium Sulphate
3. Neutral Ethyl alcohol (Neutralized with NaOH)
4. Phenolphathalein indicator
5. 0.01N NaOH

Procedure

1. Take about 10 g. of fresh muscle sample/ fish meal in a mortar & grind well with anhydrous Na_2SO_4 until all water is removed.
2. Transfer this into 250 ml. Iodine flask. Add to this 100 ml. chloroform & keep 30 min. in dark.
3. Filter the chloroform extract using filter paper and make the vol. to 100ml with chloroform.

4. Weigh 2 nos. of 50 ml conical flasks. Add 20 ml. of chloroform extract in each conical flask.
5. Evaporate the extract in water bath & then dry them for 3 hrs. in Hot air oven at 100 ^{0}C.
6. Cool and weigh the conical flask. This will give the fat content (M) in 20 ml of chloroform extract.
7. Add 10 ml. of warm, neutral alcohol & dissolve the fat.
8. Add 1 drop of phenolphthalein indicator & titrate against 0.01 N NaOH.

Calculation

$$\boldsymbol{FFA}\ (\%, \boldsymbol{as\ oleic\ acid\ on\ extracted\ fat}) = \frac{(\boldsymbol{TV\ of\ sample - TV\ of\ blank})\boldsymbol{X\ Normality\ of\ NaOH\ X\ 28.2}}{\boldsymbol{Weight\ of\ fat\ in\ chloroform\ extract}\ (\boldsymbol{20\ ml})}$$

Where

TV= Titre value

28.2 = Milli equivalent weight of oleic acid (include28.2 factor of 100 for %)

FFA content is frequently expressed in terms of acid value instead of % oleic acid. The acid value is defined as the number of mg of KOH necessary to neutralize 1 gram of extracted fat. The acid value = 2 X FFA

J. Estimation of heavy metal content in fish/ fish meal (Reference: AOAC, 1984)

Principle

The wet samples of fish are digested using Nitric acid: Perchloric acid: Sulphuric acid mixture in the ratio 3:2:1 v/v as per standard methods (AOAC, 1984) and are analysed in Atomic Absorption Spectrophotometer using air-acetylene flame with hollow cathode lamp. Standard metal solutions of AAS grade are used as reference.

Apparatus

1. Atomic Absorption Spectrophotometer connected with air-acetylene gas cylinder
2. Hollow Cathode Lamps
3. Digestion flasks

Flame AAS (Ref; AAS Manual)

The technique of Atomic Absorption developed by Sir Alan Walsh of C.S.I.R.O. in the mid 1950's has become the preferred method of elemental analysis. A light source emitting a narrow spectral line of the characteristic energy is used to excite the free atoms formed in the flame. The decrease energy (absorption) is then measured. The instrument is calibrated by standards and the sample concentrations interpolated from these. A hollow cathode lamp is used as the radiation source to excite the free atoms in the flame.

Procedure

Digestion of Sample

1. Take approx. 5 g of wet sample (0.5 g dry sample) in a 100ml Kjeldahl digestion flask.
2. Add 25 ml of acid mixture of Nitric acid: 60% Perchloric acid: Sulphuric acid (3:2:1).
3. Add a clean glass bead (acid washed).
4. Leave aside for 3-4 hrs or preferably overnight in fume cupboard.
5. Watch for foaming during the first few hrs specially with samples containing high amount of fat.
6. If foaming occurs and threatens to overflow; immerse the bulb of flask in cool water till froth subsides.
7. Heat for 30 min., then more strongly for 4hrs. till clear the solution.
8. If charring occurs or flask become dry, remove from heat, cool & add 5 ml of conc. HNO_3 & then continue heating.
9. Digested sample diluted to 25 ml with glass distilled water.
10. Carry a blank with each set of sample by digesting 25 ml dist. water + 25 ml acid mixture, diluted to 25 ml.
11. It is then aspirated over the flame in AAS for determining the concentration of the metals.

Calculation

Heavy metal concentration is expressed in ppm (parts per million).

K. Estimation of histamine content in fish meal

Scombroid fish contains plentiful amount of histidine. Spoilage bacteria act on histidine forming histamine. Histamine is suggested as a possible index of spoilage.

Chemicals:

1. **p-phenyl diazonium sulfonate: Chilled 1.5 ml 0.9% (w/v) sulfanilic acid in 4% HCl and 1.5% 5% sodium nitrite** (Mix both in 50 ml standard volumetric flask and keep in ice bath for 5 min. Add 6 ml more of 5% sodium nitrite. Allow it to stand for 5 min. and make up the volume with chilled distilled water. Store the reagent in ice bath and use after 15 min. after dilution with water. Reagent is stable for 12 hours only.)
2. **Salt mixture:** Mix 6.25 g anhydrous sodium sulphate and 1 g tri-sodium phosphate monohydrate

Procedure

Muscle Extract Preparation

1. Weigh 5 g of fish mince in 50 ml centrifuge tube
2. Add 20 ml 0.85% sodium chloride (NaCl) solution (Saline) and homogenize for 2 min.
3. Centrifuge at 12000Xg (7500 rpm) for 15 min at 4 degree Celsius
4. Collect the supernatant and make upto 25 ml with saline
5. Muscle extract can be used immediately for analysis

Analysis

1. Take 1 ml of the muscle extract into 15 ml of centrifuge tube
2. Add 2 ml saline and 0.5 g of salt mixture

3. Shake thoroughly and add 2 ml of n-butanol and shake vigorously for 1 min.
4. Allow the same to stand for 1 min.
5. Shake again to break the protein gel and shake further for few second with vigorous action
6. Centrifuge at 3100Xg (7500 rpm) for 10 minutes
7. Take one ml of upper butanol layer in a clean dry test tube and evaporate under Nitrogen gas
8. Dissolve the residue with 1 ml of Double distilled water
9. In a clean dry test tube, take 5 ml of 1.1% sodium carbonate solution and add 2ml of chilled reagent slowly. Mix it well.
10. Add above reagent to the dissolved residue and allow it to stand for 5 min.
11. Measure the absorbance at 496 nm.
12. For blank use 1 ml Distilled water instead of dissolved residue.

For standards

1 ml aliquot of standard histamine solutions from 0-100 microgram/ml can be reacted with the reagent mix. Check the absorbance and prepare the standard curve of absorbance against histamine concentration.

Formula for Estimation of histamine concentration in the sample

$$\boldsymbol{Histamine\ (mg/100g) = \frac{A\ X\ 2\ X\ 25\ X\ 100}{5\ X\ 1000}}$$

A is the value of histamine obtained in microgram per ml from the standard curve.

L. Estimation of salt content in fish/ fish meal

Principle

Sodium chloride on reaction with silver nitrate forms silver chloride which is estimated by titration with ammonium thiocyanate in the presence of ferric alum indicator.

Chemicals

1. 0.1 N silver nitrate
2. 0.1 N ammonium thiocyanate
3. Ferric alum indicator (140 g of ferric ammonium sulfate crystals in 400 ml of hot water. Cool it, filter and make up to 500 ml with dilute 6N nitric acid)
4. Nitric acid

Procedure

1. Take 1 g of dry sample or 5 g of fresh sample in 250 ml conical flask. Add 20 ml double distilled water, 5 ml of concentrated nitric acid and 40 ml of 0.1 N silver nitrate
2. Boil the same on hot plate for 15 minutes
3. Cool and titrate against 0.1 N ammonium thiocyanate using 1 ml of ferric alum indicator till brick red colour appears. Note down the end point.

1 ml of 0.1 N $AgNo_3$ = 0.0058 g of NaCl

$$\textbf{\textit{Salt content}}\ (\%) = \frac{(\textbf{\textit{Titre value of blank}} - \textbf{\textit{Titre value of sample}})\textbf{\textit{X}}\ \mathbf{58.45}}{\textbf{\textit{Weight of sample X}}\ \mathbf{100}}$$

M. Estimation of acid insoluble ash content (sand silica) of fish meal

Apparatus

1. Silica crucible
2. Muffle furnace
3. Ashless filter paper (Whatman Filter Paper No. 41/ 42)
4. Analytical balance

Chemicals

1. HCl (2 N)

Procedure

1. Take weight of empty crucible (W_1).
2. Add 5 g of moisture free dry sample.
3. Take weight of crucible with sample (W_2).
4. Keep it in muffle furnace for ashing at 550 degree celsius for ashing.
5. Take weight of crucible with ash (W_3).
6. Calculate ash content on dry weight basis in %.
7. Add 25 ml of dilute HCl (2N) to the crucible with ash
8. Boil it for 5-7 minutes on hot plate
9. Let it cool and then filter it using ashless filter paper. Give washing to the crucible with hot water. Wash the residue on the filter paper with hot water till whole acid is washed off.
10. Discard the filtrate. Fold the filter paper and keep it in pre-weighed crucible.
11. Keep it for ashing in muffle furnace at 550 degree Celsius.

12. Take weight of crucible after cooling.
13. Calculate acid insoluble ash.

$$\textbf{\textit{Acid Insoluble Ash}}\ (\%) = \frac{(\textbf{\textit{Weight of crucible + ash}}) - (\textbf{\textit{Weight of empty crucible}})}{(\textbf{\textit{Initial weight of crucible + sample}}) - (\textbf{\textit{Weight of empty crucible}})}\ \textbf{\textit{X}}\ \mathbf{100}$$