

APPENDIX

Determination of protein

Kjeldahl method was used in this experiment for determination of protein content. The sample (1 g.) was detected with 20 ml of concentrated sulfuric acid (H₂SO₄) and 5 g of catalysts (10:1 K₂SO₄:CuSO₄) at 380°C until digestion was completed (turn into clear solution). For distillation step, the 100 ml of 40% NaOH was used to react with (NH₄)₂SO₄ from digest step. The NH₃ was collected by 25 ml of 3% boric acid. Then, the solution was titrated with 0.1M of HCl to determine the concentration of NH₃. The protein content is calculated as follows:

$$\% \text{ Total nitrogen} = \frac{(A-B) \times C \times 0.014 \times 100}{D}$$

Where A; Vol. of HCl (ml) used in the sample titration

B; Vol. of HCl (ml) used in the blank titration

C; Normality of the standard acid

D; Weight of sample (g.)

% protein content = % total nitrogen x factor

Where Factor; 5.46

Determination of moisture content

The aluminum can was heated at 105°C and weighed. The 1 g of sample was added in aluminum can and re-weighed. The aluminum can was heat at 105°C until the weight was constant. The moisture content is calculated as follows:

$$\% \text{ moisture} = \frac{\text{wet weight} - \text{weight after drying}}{\text{wet weight}} \times 100$$

Determination of ash

The crucible was heated in a furnace at 550°C and weighed (W1). The 5 g of sample was added (W2) in crucible and placed in the furnace. The crucible was heated at 550°C overnight and re-weighed (W3). The percentage of ash is calculated as follows:

$$\% \text{ ash} = [(W3 - W1)/W2] \times 100$$

Determination of fat

Goldfish extraction was used for determined fat. The 2 g of sample was added in the trimble. The sample was extracted by 80 ml of petroleum ether at 150°C for 2.30 h. The defatted sample was re-weighted.

Determination of crude fiber

The 1 g of defatted sample was digested in 150 ml of hot 1.25% sulfuric acid for 30 min followed with 150 ml of hot 1.25% sodium hydroxide for 30 min. The sample was washed with 95% of ethanol. The sample was putted in the crucible and heated at 105°C until constant weight. The crucible was placed in furnace at 500°C for 2 h.

Determination of Total starch

The sample was weighed in 100 mg and added into the tube. The 0.2 mL of 80%(v/v) ethanol was added in the sample and mixed by using vortex mixer. Then the 2 mL of cold 2 M potassium hydroxide was added in the mixture. The tube was stored in the refrigerator at 4°C for 24 h. The 8 mL of 600mM sodium acetate buffer (pH 3.8), 0.1 mL thermostable α -amylase (3,000 U), and 0.1 mL of AMG (3,300 U) were added and mixed by using vortex. The mixture was incubated in water bath at 50°C for 30 min and the volume of sample was adjusted to 100 mL before determine the free glucose by PGO enzyme reagent, To determined the free glucose by PGO enzyme reagent, the 2.5 mL of PGO enzyme reagent was added in 0.05 mL of diluted sample and mixed with 0.2 mL of distilled water, then incubated at 37°C for 30 min. The absorbance of the solution was measured at 440 nm.