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APPENDICES

APPENDIX I

Woody Plant Medium (WPM; Lloyd and Mc Cown, 1980)

Constituents

Inorganic Compound		
NH_4NO_3	400	mg/l
$CaCl_2.2H_2O$	96	mg/l
$MgSO_4.7H_2O$	370	mg/l
KH_2PO_4	170	mg/l
$Ca(NO_3)_2.4H_2O$	556	mg/l
K_2BO_3	990	mg/l
$MnSO_4.4H_2O$	22.30	mg/l
H_3BO_3	62	mg/l
$Na_2MoO_4.2H_2O$	0.25	mg/l
$CuSO_4.5H_2O$	0.25	mg/l
Na_2EDTA	27.80	mg/l
$FeSO_4.7H_2O$	27.80	mg/l
Vitamin		
Inositol	100	mg/l
Nicotinic acid	1.0	mg/l
Pyridoxine HCl	0.5	mg/l
Thiamine HCl	0.5	mg/l
Glycine	20	mg/l
Agar	8	g/l
Sucrose	30	g/l

pH 5.6

APPENDIX II

Proximate analysis (AOAC, 1995)

1. Starch analysis

Polarimetric Method-The European Economic Community

1.1 Apparatus

- Analytical balance
- Volumetric flask 100 ml. and 250 ml
- Polarimeter
- Funnel Ø 65-70 mm.
- Boiling water bath
- Filter paper (Whatman No.1 and 42)

1.2 Reagents

- Hydrochloric acid solution, 1.128% and 25% HCl
- Sodium Phosphotungstate or Dodeca Phosphotungstic acid 4%

1.3 Determination

1.3.1 Sample preparation

The sample was grinned and passes though No. 20 Mesh sieve. Five g of sample was weighed and added into 200ml flask with the aid of the funnel. The 50

ml of 0.3904 N conc. HCl were added into the flask that was shaken gently till the sample was completely wet. Thereafter the 50 ml of 0.3904 N conc. HCl were re-added. The flask was placed in boiling water bath and shaken for 3 minutes for anti-precipitation. During shaking, the flask had to be placed in boiling water bath for exactly 15 minutes. The flask was transferred and 60 ml cool water was added then cooled to 20°C immediately. The 20 ml of Sodium Phosphotungstate or Dodeca phosphotungstic acid were added and shaken strongly for precipitating. Then 200 ml of H_2O were added and shaken well till the solution was completely dissolved. The solution was filtered though the filter papers No.1. The first 25 ml of filtrate was dispersed and then the second filtrate was collected into the polarimeter tube.

The total rotatory power (P), angular degree, at 20°C. Before the observation, it had to test for the precipitation by adding a drop of Sodium Phosphotungstate or Dedeca Phosphotungstic acid in 2 ml of the filtrate solution and allowed standing for 3 minutes. Re-analysed if the solution became turbid by increasing the amount of Sodium Phosphotungstic or Dedeca Phosphotungstic acid.

1.3.2 Analysis of rotary power of active water-soluble substance after reacted with HCl (P')

Two point five g of sample was added into the 250 ml flask. Two hundred ml distilled water were added followed by shaking till the sample was complete wet. The flask was allowed to cool at room temperature for 1 hour, with shaking approximately 6 times then added the 250 ml of H_2O with gently shaking. The solution was allowed for the starch precipitating and then filtered through Whatman filter paper No.42. The filtered solution containing no starch tested by

Iodine solution. The 10 ml of solution (as 5.0g sample) were pipetted into the 200 ml flask and then 4.2 ml of 2.5% HCl was added, shaken well. The flask with the aid of the funnel was transferred to the water bath for 15 minutes, rotating shaken for 15 minutes. The flask was removed and 60 ml of cooled water was added then cooled to 20 °C. Sodium Phosphotunstate or Dedeca phosphotungstate was added as above. Two hundred ml of water at 20 °C was added into the flask, which was shaken gently. The solution was then filtered till the solution was cleared (left the first 25 ml of solution). The P' value of filtered solution was observed.

1.4 Calculation

Let

A = % Starch

P = Total rotatory power, angular degree

P' = Rotatory power of active water-soluble substance, angular degree

M = % Humidity

$\{\alpha\}_D^E$ = specific rotation of starch angular degree

Then

$$A = \frac{2000 \times (P-P') \times 100 \times 2}{\{\alpha\}_D^E \times (100 \times M)}$$

Specific rotation of Starch

185.9 ° : rice starch

195.4 ° : potato starch

184.6 ° : maize starch

182.7 ° : wheat starch

181.5 ° : barley starch

181.3 ° : oat starch

184.0 ° : other types of starch and also starch mixtuers in compound feeding stuff

2. Fat analysis (AOAC., 1995)

Soxhlet Method

2.1 Apparatus

- Extraction apparatus : continuous, for example the soxhlet type with and extraction flask of 150 ml. capacity
- Extraction thimbles
- Heating units : water bath or steam bath

2.2 Reagents

- Petroleum ether : boiling range 40-60 °C

2.3 Determination

2.3.1 Preparation of the extraction flask

The extraction flask was dried in air oven, preheating 102±3 °C, for 2 hrs. The flask was cooled in a desiccator at room temperature and weighed. The

process were repeated until the results of two successive weightings did not differ by more than 1 mg The least weight of flask (X) was recorded.

2.3.2 Analysis of fat

The dried sample was grinded and weighed for approximately 5 g in a thimble (W). The thimble was placed in the extraction tube and connected with the weighed flask containing 100 ml Petroleum ether. The extractor was connected with a condenser tube. The flask was heated at constantly temperature, the solution was siphoning 5-6 times per hour and then continued the extraction for 6-8 hours. The thimble was removed from the extraction tube and the Petroleum ether extract was evaporated to dryness not over 100 °C. The sample was grinded and poured into thimble and then extracted for 2 hours. The Petroleum ether extract was evaporated to dryness on water bath until the weight was constant (Y)

2.4 Calculation

Let

W : Weight (g) of the dried sample

Y : Weight (g) of the flask and extracted sample

Z : Weight (g) of the extraction flask

Then

$$\text{Total Fat (\%)} = \frac{100\,[\,W-(Y-X)]}{W}$$

3. Fiber analysis (AOAC., 1995)

3.1 Apparatus

- Digestion apparatus : with condenser to fit 600 ml beaker
- Filtration apparatus : Filter paper No.1 and No 42
- Gooch crucible of Alundum crucible R-98
- Desiccator : with efficient desiccant
- Oven
- Furnace

3.2 Reagents

- Sulfuric acid 0.255 N.
- Sodium hydroxide 0.255 N.
- 95% Ethanol

3.3 Determination

Twopoint five g of dried sample was transferred into a 600 ml beaker. Two hundred ml of boiling 0.255 N Sulfuric acid were added into the beaker and then placed on a digestion apparatus, allowed to heat till 30 minutes. The solution was then filtered through a filter paper No.1 using suction and then washed with the hot water (approximately 200 ml) until acid-free (tested by pH paper). The filter paper containing all insoluble matter was placed to the beaker. The 200 ml of boiling 0.313 N NaOH was added into the beaker and then the beaker was placed on the digestion apparatus, heated till 30 minutes. The solution was once filtrated through the filter

paper No. 42 using the suction and then washed with hot water (approximately 200 ml) until base-free (tested by pH paper). The paper was washed with 10 ml 95 % Ethanol and then removed to a crucible and dried for 2 hours in oven (Memmert ULM. 500m Germany) at 105-110 °C. The crucible was cooled in a dessicator, weighed and re-dried for 30 minutes until the results of two successive weightings did not differ by more than 1 mg. The least weight (W1) was recorded. The crucible with containing fiber was ignited in the furnace (Thermolyne 47900, USA) 30 minutes at 600 ± 15 °C., cooled in the desiccator and weighed. The crucible was re-ignited 30 minutes until the results of two successive weightings did not differ by more than 1 mg. The least weight (W2) was weighted.

3.4 Calculation

Let

W : Weight (g) of sample

W1 : Weight (g) of crucible and insoluble matter

W2 : Weight (g) of crucible and ash

Then

$$\% \text{ Crude Fiber} = \frac{00\,(\,W1\text{-}W2\text{-}\,\text{fiber paper weight})}{W}$$

4. Protein analysis (AOAC, 1995)

4.1 Apparatus

-Kjeldahl flask : capacity about 800 ml. provided, if desired with a pear shaped glass bulb loosely fitting into the flask

- Distillation apparatus : steam or direct
- Heating device

4.2 Reagents

- NaOH
- Bori
- Anhydrous Sodium carbonate
- Bromocresol green
- Methyl red
- 95% Methanol
- conc. Sulfuric acid
- conc. Hydrochloric acid
- catalysts (7 g. K_2SO_4 + 0.8 g $CuSO_4.5H_2O$)
- distilled water or deionized water

4.3 Detemination

4.3.1 Reagents preparation

- Sodiumhydroxide solution: 400 g of NaOH were dissolved in water and diluted to 1000 ml.
- Sodium hydroxide solution 1 mol/l
- Sodium hydroxide solution 0.1 mol/l
- Bromocresol green solution: 0.1 g of Bromocresol green were dissolved in the 100 ml. of Ethanol.

- Methyl red solution: 0.1 g of Bromocresol green and Methyl red was dissolved in 100 ml. of Ethanol.
- Mixed indicator solution: 0.1 g of Bromocresol green and Methyl red was dissolved in 100 ml. of Ethanol.
- Boric acid solution.

Four hundred g of Boric acid was dissolved in approximately 6 l. of distilled water then boiled on hot plate, swirled till completely dissolving. The solution was adjusted to 9 l with hot distilled water.

The solution was allowed to cool to the room temperature. Then the 100 ml of Bromocresol green solution and 70 ml of Methyl red solution were added respectively. The solution was added to 10 l. with distilled water and swirled to mix.

Twenty five ml of Boric acid solution was pipetted in flask and then added with 100 ml of distilled water. The solution was titrated with 0.1 mol/l NaOH until the color changed to red-violet. Calculating the amont of used 1 mol/l NaOH for 10 litre by criteria following

ml of 1 mol/l NaOH = ml of mol/1 NaOH

The amount of 1 mol/l NaOH was added into Boric acid solution and swirled well.

- Hydrochloric acid, 0.1 mol/l HCl, standardised

Eight point two ml of conc. HCl was pipetted and diluted with distilled water to 1000 ml.

Standardised : 5 g of anhydrous Na_2CO_3 was grinded, dried at 265°C for 1 hour or at 200 °C for 2 hours and allowed to cool in desiccator

Point thirteen g of dried Na_2CO_3 (above) was added into a flask and the 20 ml of distilled water was added. Five drops of indicator was mixed and then titrated with Hydrochloric acid solution until color changed to pink.

The flask was removed into the water bath and boiled for 2-3 minutes. The flask was cooled to the room temperature (violet solution) then the solution was titrated with Hydrochloric acid until the color changed to pink. The amount of used HCl (A2) was recorded. Calculating for the concentration of HCl solution by the following equation

$$HCL](mol/l) = \frac{2000 \times \text{exactly weight of } Na_2CO_3}{(A1+A2) \times \text{MW of } Na_2CO_3}$$

4.3.2 Analysis of protein

Point five g of sample was added into a digestion tube. Seven g of catalyst and the 10-15 ml of conc.H_2SO_4 were added respectively. The digestion tube was transferred to the digester (Tecator digestor 1006, Sweden) at 420°C until the solution was cleared then removed from the digester and allowed to cool. A flask containing 25 ml of 4% Boric acid and the digestion tube were transferred into a distillator (Tecator Kjeltec system 1026, Sweden). The solution was titrated with standardized HCl until the solution turned to pink.

4.4 Calculation

Let

f : 6.25 for general factor

Then

$$\%N = \frac{14.001 \times [HCl] \times (\text{volume ot HCl with sample} - \text{volume of HCl with blank}) \times 100}{\text{weight of sample (mg.)}}$$

$$\%\text{Protein} = \%\ N \times f$$

5. Ash analysis (AOAC, 1995)

5.1 Apparatus

- Porcelain crucible
- Furnace : Thermostatically controlled at 600 ± 20 °C (Thermolyne 4790, USA)
- Desiccator
- Hot plate

5.2 Determination

A porcelain crucible was placed into the temperature-controlled furnace, preheated to 600 ± 20 °C for 1 hour then transferred directly to desiccator for cooling and weighed immediately. The crucible was re-ignited for 30 minutes., cooled in the desiccator and weighed. The process 5.2.1-5.2.2 were repeated until the results of two successive weightings did not differ by more than 1 mg. Five g. (W1) of sample was added into the crucible. Then it was placed on a hot plate under a fume-hood and slowly increased the temperature until the smoking ceased. The crucible was placed inside the furnace at 600 ± 20 °C for 2-3 hrs till the sample became thoroughly ash. Then the crucible was removed from the desiccator to cool and weighed (W2).

5.3 Calculation

Let

W : Weight (g) of porcelain crucible

W1 : Weight (g) of sample

W2 : weight (g) of crucible and sample

Then

$$\text{Ash (\%)} = \frac{100\,(W2-W)}{W1}$$

BIOGRAPHY

Miss Rattana Panriansaen was born on January 18, 1973 in Khon Kaen Province, Thailand. She received her Bachelor of Science in Biology, Faculty of Science, Khon Kaen University in 1995, and graduated with a Bachelor of Arts in Political Science, Faculty of Political Science, Ramkamhaeng Univesity in 1996. She has studied for Master's Degree in Biotechnology program at Chulalongkorn University since 1997.