Management Procedures for Evaluation of Pearl Millet and Sorghum Grain Quality

Compiled by

T. Nagur, V. Subramanian, and D.L. Oswalt

Skill Development Series no. 11

ICRISAT

Human Resource Development Program

International Crops Research Institute for the Semi-Arid Tropics
Patancheru, Andhra Pradesh 502 324, India

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Contents

Introduction

Pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) are the cereals being improved at ICRISAT. The improvement in related skill development involves collection and conservation of genetic material, evaluation of high-yielding genotypes with good quality grain and resistance to abiotic and biotic stresses, and the development of agronomic practices to increase their grain yield. Individualized skill development programs are provided to improve field and laboratory techniques.

MP 1. Breaking Dormancy in Pearl Millet

Most of the cultivated varieties of pearl millet seed do not show post-harvest dormancy and germinate on the earhead when continuous rains are received after physiological maturity of the seed. However, some varieties show post-harvest dormancy that can be broken:

a. by placing the seed in contact with a blotter soaked with 0.2% KNO_3 solution and incubating alternately at 5°C and 35°C in darkness for 28 days (Anderson 1958).

b. by soaking in a solution containing 1% 2-chloroethanol and 0.5% potassium hypochlorite for 1 h (Burton 1969).

c. by keeping the water-soaked seed in an incubator maintained at 35°C for 24 h.

d. by dusting the seed with 5% 'Ethephon®'.

MP 2. Moisture Estimation

Moisture content of a flour sample is determined by drying overnight at 100°C or at 130°C for 2 h in an oven to constant mass (Association of Official Analytical Chemists 1984).

Equipment

Hot-air oven to maintain a temperature from 100°C to 130°C and aluminum-moisture dish.

Procedure

1. Weigh an empty aluminum-moisture dish (W_1) and place about 2.5 g of flour into the dish and weigh (w_2).

2. Spread the flour evenly, place without lid in an oven and dry the samples overnight at 100°C or 2 h at 130°C.

3. Place lids on the dishes and place in a desiccator to cool, charged with silica-gel crystals.

4. Continue drying and cooling until a constant mass is obtained.

5. Obtain the constant mass of the aluminum dish containing the sample after cooling (W_3).

Calculation

$$\text{Moisture } (\%) = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Example

W_1 = Mass of aluminum dish = 15.2525 g
W_2 = Mass of dish + flour sample = 17.9050 g
W_3 = Mass of dish + flour after drying to constant mass and cooling = 17.6425 g

$$\text{Moisture } (\%) = \frac{(17.9050\ g - 17.6425\ g)}{17.9050\ g - 15.2525\ g} \times 100 = 9.8\%$$

MP 3. Oil Estimation (Soxhlet)

Oil is extracted from a sample with a Soxhlet apparatus using hexane. The extracted oil is separated by evaporation of the hexane for its estimation (Official and Tentative Methods of the American Oil Chemists' Society 1981).

Equipment

Balance, Soxhlet apparatus, sand bath, oven, and fume hood.

Reagent

Hexane

Procedure

1. Weigh 3-4 g of the moisture-free-flour sample on a Whatman No. 2 filter paper. (Moisture in the sample is reduced by drying the sample in an oven overnight at 100°C or 2 h at 130°C) .
2. Enclose the weighed sample in a second filter paper folded like a thimble.
3. Insert this into a Soxhlet thimble. Place a piece of absorbent cotton in the top of the thimble to distribute the solvent as it drops on the sample.
4. Place the thimble into a Soxhlet extraction tube and assemble the Soxhlet apparatus.
5. Pour hexane into the extraction flask, up to 3/4 of its volume (about 170 mL) .
6. Heat on the mantle of the Soxhlet apparatus at such a rate that 150 drops of the solvent fall on the center of the thimble min^{-1}.

   Note: A continuous and regulated water supply should be ensured through the apparatus.
7. Keep the volume of solvent in the extraction flask fairly constant to make up the loss, if any due to evaporation.
8. Continue extraction for 18 h.
9. Cool and disconnect the extraction flask.
10. Transfer the contents of the extraction flask with repeated washings of hexane into a clean dry, previously weighed 250 mL beaker.
11. Evaporate the hexane on a hot-sand bath in a fume hood.

    Note: Complete evaporation of the hexane must be ensured, before placing the beaker containing the extracted oil into an oven for drying.
12. Dry the beaker in an oven at 105°C for 30 min to evaporate all traces of hexane.
13. Cool the beaker in a desiccator and weigh.

Calculation

Mass of the beaker + Oil = A
Mass of the beaker = B
Mass of the sample = C

$$\text{Oil \% in the sample} = \frac{(A-B) \times 100}{C}$$

Example

Mass of the beaker + oil = 84.045 g
Mass of the beaker = 83.936 g
Mass of the sample = 3.587 g

$$\text{Oil \% in the sample} = \frac{(84.045\ g - 83.936\ g) \times 100}{3.587\ g} = 3.0\%$$

MP 4. Protein Estimation

a) Microkjeldahl method

The nitrogen in a grain sample is converted to ammonium sulfate by digestion with sulfuric acid at 380°C in the presence of catalysts, potassium sulfate, and mercuric oxide. The ammonia is liberated by distilling the digest with sodium hydroxide solution and was absorbed in boric acid solution. It is titrated with a standard acid.

Equipment

Balance, Microkjeldahl (Mkj) digestion set, and Mkj distillation set.

Reagents

a. **Digestion mixture (catalyst).** Thoroughly mix 190 g anhydrous potassium sulfate and 4 g mercuric oxide.

b. **Concentrated sulfuric acid.**

c. 60% sodium hydroxide solution. Dissolve 600 g sodium hydroxide and 50 g sodium thiosulfate in distilled water, cool, and make up the volume to 1 L.

d. **Boric acid.** Dissolve 40 g of boric acid in distilled water and make up the volume to 1 L.

e. **Double indicator.** Dissolve separately 200 mg methyl red and 200 mg bromocresol green in 100 mL of 70% ethyl alcohol. Mix one part methyl red with five parts bromocresol green to make the indicator solution.

f. **Hydrochloric acid (0.02 N HC1).** Add 8.5 mL of concentrated hydrochloric acid to 5 L of distilled water. Standardize to get 0.02 N acid by titrating against a standard sodium carbonate (0.02 N) solution.

Procedure

1. Weigh about 100 mg of a finely ground sorghum or pearl millet sample into a Microkjeldahl digestion flask.
2. Add about 2 g of the digestion mixture **(Reagent a).**
3. Dispense 2 mL of concentrated sulfuric acid **(Reagent b)** into the flask.
4. Digest in a Mkj digestion set till the solution becomes clear (for about 1 h). Cool the clear mixture.
5. Dissolve the digest with a minimum quantity of distilled water and transfer to a Mkj distillation set.
6. Add 10 mL of sodium hydroxide solution **(Reagent c)** and distill.
7. Collect the distillate in 5 mL of boric acid **(Reagent** d) containing 2 drops of double indicator **(Reagent e)** in a 50 mL conical flask until the color changes in the solution.
8. Titrate the distillate against a standard hydrochloric acid **(Reagent** f) and record the mL of titrate used as the titer value (TV).

Calculation

$$N\ (\%) = \frac{\text{(normality of HC1) x (TV in mL) x Atomic mass of N x 100}}{\text{Sample mass (mg)}}$$

The atmoic mass of N = 14.007.

Nitrogen (%) is converted into protein by multiplying by a factor of 6.25.

Example

Normality of HC1 = 0.02 Normal (N)
Titer value (TV) = 4.82 mL
Atomic weight of nitrogen = 14.007
Sample mass = 95.3 mg

$$N\% = \frac{0.02\ N \times 4.82\ mL \times 14.007 \times 100}{95.3\ mg} = 1.417\%\ N$$

Protein (%) = N(%) x 6.25 (factor)
= 1.417% N x 6.25 = 8.86%

b) Technicon-autoanalyzer method

The quantitative determination of protein by a Technicon Autoanalyzer (TAA) involves the conversion of organic nitrogen into ammonia by digesting a sample with a mixture of sulfuric acid and orthophosphoric acid. Ammonical nitrogen in the digest reacts with sodium phenate in the presence of acidified sodium hypochlorite to form an indo-phenol blue complex. The color complex is measured at 660 nm (Industrial Method 1972).

Equipment

Balance, block digestor (BD 40), and Technicon Autoanalyzer

Reagents

a. **Acid mixture.** Five parts (v/v) orthophosphoric acid in 100 parts of sulfuric acid.

b. **Kjel tabs auto.** Each tablet contains 1.5 g K_2SO_4 containing 7.5 mg selenium.

c. **Alkaline sodium potassium tartrate.** Dissolve 75 g sodium hydroxide and 50 g sodium potassium tartrate in about 900 mL of distilled water, cool and dilute to 1 L.

d. **Alkaline phenol.** Mix 138 mL of phenol (88%) with 500 mL of 5 N NaOH in an ice bath; make up to 1 L with distilled water.

e. **Sodium hypochlorite (NaOCl).** Dilute commercially available bleach to get 5% NaOCl, if necessary.

f. **4% sulfuric acid.** Add 40 mL of concentrated sulfuric acid into about 800 mL distilled water and make up to 1 L.

g. **Ammonium sulfate standards.** Dissolve 4.717 g of oven-dried ammonium sulfate in 1000 mL distilled water (1000 pprn N stock solution). Take 5, 10, 15, 20, and 25 mL of stock solution into 500 mL volumetric flasks and make up their volumes with 4% sulfuric acid. Label the solutions as 10, 20, 30, 40, and 50 ppm nitrogen respectively.

Procedure

1. Weigh about 100 mg flour and transfer it to a Technicon digestion tube (75 mL).

2. Add 3 mL of acid mixture (Reagent a) and 1 Kjel tab (Reagent b) into the tube.

3. Arrange a set of 40 tubes and fix the tubes with supports and clamps. Let each set of 40 tubes consist of a blank, control standard, and 38 unknown samples.

4. Keep the set on the block digester and digest it for 1 h at about 375°C.

5. Cool the digest and mix it with a minimum amount of water.

6. Make up the volume to 75 mL mark in the tube, close the tube with a stopper and thoroughly mix the solution.

7. Transfer an aliquot from each tube into a Technicon sample cup and load the rotating disc of the Technicon sampler.

Calibration

1. Run alkaline sodium potassium tartrate (Reagent c), alkaline phenol (Reagent d), sodium hypochlorite (Reagent e), and wash solution through the respective tubings for a minimum of 15 min.

2. Place the ammonium sulfate standard solutions in sample cups in the rotating disc. Run standards by switching 'on' the sampler.

3. Calibrate the instrument with 50 ppm nitrogen solution to give 90 divisions on a TAA chart by using the base line and calibration knob. Verify if 10, 20, 30, and 40 ppm nitrogen solutions give the correct net division values (for example 10 ppm should give 18 net divisions, i.e., 1 ppm is equivalent to 1.8 net divisions).

4. Place the rotating disc containing sample digests on the autoanalyzer and run by switching 'on' the rotating disc.

5. Record the sample-peak heights (net divisions from the graph).

Calculation

$$N\ (\%) = \frac{\text{Net divisions for sample aliquot} \times \text{Made-up volume (mL)} \times 100}{1.8\ \text{(net division for 1 pm standard)} \times 1000 \times \text{Sample mass (mg)}}$$

Calculate protein (%) by multiplying N (%) by the conversion factor 6.25.

Example

Sample net division = 50 ND
Standard net division = 1.8 ND
Sample mass = 95 mg
Made up volume = 75 mL

$$N\ (\%) = \frac{50\ (ND) \times 75\ mL \times 100}{1.8\ (ND) \times 1000 \times 95\ mg} = 2.19\%$$

Protein (%) = 2.19% x 6.25 = 13.7%

c) Dye binding capacity method

Protein content in cereals (sorghum and pearl millet) can be determined by the amount of the dye (acid orange 12) bound to the basic amino acids in protein. The dye binding capacity (DBC) method correlates (r=0.90) well with the standard Microkjeldahl procedure. A regression equation established between the Udy instrument reading (UIR) and the protein content determined by the standard Microkjeldahl procedure can be used to estimate protein percentage (Udy 1956; Greenaway 1972; and Jambunathan et al. 1983) .

Equipment

Balance, Udy mill, Udy shaker, Udy analyzer, Udy bottles, Udy filter set, dye dispersion kit, and other equipment as in section MP 4(a).

Reagents

a. **Reagent dye.** Dilute one bottle of reagent dye concentrate (SL-1215) to 20 fold with distilled water and thoroughly mix. The resulting dye concentration is 1.3 mg mL^{-1}.

b. **Reference dye.** Dilute the reference dye concentrate of one bottle (SL-1513) 20 fold with distilled water to obtain a concentration of 0.6 mg mL^{-1}.

Procedure for sorghum

1. Switch on the Udy analyzer and allow it to warm for 2 h. Using a reference dye adjust the Udy instrument reading (UIR) to 42% transmission (T).

2. Weigh approximately 1.0 g of cereal flour into a Udy bottle. Add 40 mL of reagent dye. Mix the contents for 1 h in a reciprocating shaker (Udy).

3. Check the reagent dye concentration (Udy calibration kit: SL-1511) along with the diluted reagent dye. The readings follow an exponential curve, as given in the following example.

Dye conc (mg mL^{-1})	0.35	0.45	0.65	0.75	0.85
UIR (Transmission %)	79.0	60.5	36.5	29.0	22.5

4. a) Assemble the filter and place it on the flow-through cuvette (Udy analyzer).

   b) Pour the contents of the Udy bottle (from step 2) into the filter. Record the Udy instrument reading (Transmission percentage).

Standardization and calculation of the regression coefficient (m) and the intercept (C).

- Select 20 cultivars with a wide grain-protein range and analyze them in duplicate by the Microkjeldahl method (refer section MP 2a).
- Carry out the DBC method of analysis on duplicate sets of the same 20 cultivars and calculate the mean UIR.
- Plot the mean UIR values against the Microkjeldahl-protein values and draw the best fit line.
- Compute the regression coefficient (m) and intercept (C) by following the method of regression analysis to predict the estimated protein percentage.

Estimated protein (%) = (m x UIR) + C

Where, m is the regression coefficient and C is the intercept from the regression equation.

Example:

m = 0.315 UIR = 32% C = 1.05

Estimated protein (%) = (0.315 x 32%) + 1.05 = 11.1%.

MP 5. Lysine Estimation

The lysine percentage of protein can be estimated by the dye binding capacity method using the ratio between the Udy instrument reading (UIR) and protein percentage (Udy 1971; Jambunathan et al. 1983) .

Equipment

Balance, Udy mill, Udy analyzer, Udy bottles, Udy shaker, dye dispersion kit, Udy filter sets, and other equipment as in section MP 4 (a).

Reagents

Refer section MP 4(c).

Procedure

1. Weigh 1 g of ground cereal sample into a Udy bottle.
2. Add 40 mL of dye solution (Acid orange-12 concentration 1.3 mg mL^{-1}).
3. Mix the contents in a reciprocating shaker (Udy shaker) for 1 h.
4. Switch 'on' the Udy analyzer and warm it for 2 h. Adjust the Udy instrument reading (UIR) to 42 (%T) with reference dye.
5. Check the standard dye concentration (Udy calibration kit: SL-1511) along with the half diluted reagent dye. They follow the exponential curve.

Dye conc. (mg mL^{-1})	0.35	0.45	0.65	0.75	0.85
UIR (T%)	79.0,	60.5,	36.5,	29.0,	22.5

6. Assemble filter and keep it on the flow-through cuvette. Pour the contents of the bottle (from step 3) into the filter. Note the Udy instrument reading (transmission percentage).
7. Read the percent transmittance (UIR) of the filtrate using the flow-through cuvette and Udy colorimeter.
8. Determine the protein content of the sample by the method given in MP 4(a).
9. Calculate the UIR unit'[1] protein (P) and use the following regression equation to estimate lysine.

$$\text{Estimated lysine [g (100 g Protein)}^{-1}\text{]} = m\ (UIR/P) + C$$

Where, m is the regression coefficient
C is the intercept from the regression equation
P is the protein content in the sample

Regression Equation

1. Select about 20 cultivars with a wide lysine range of the grain population. Determine their lysine contents using an amino acid analyzer.
2. Determine protein in the samples in duplicate by the microkjeldahl method given in section MP 4(a).
3. Carry out the DBC analysis in duplicate for the same samples and calculate the mean UIR. Calculate UIR/P ratio.

4. Plot the mean UIR/P values against the lysine values and draw the best fit line.

5. Compute the regression coefficient (m) and intercept (C) to predict the lysine content.

6. Report the estimated lysine as grams $(100\ g)^{-1}$ of protein.

Estimated lysine % of protein = m (UIR/P) + C

Example

Protein content of sample (P%) = 7.9
Udy instrument reading (UIR) = 28.0
Regression coefficient (m) = 0.582
Intercept (C) = 0.152

Estimated lysine (%) = m (UIR/P) + C

Estimated lysine (%) = 0.582 x (28.0 ÷ 7.9) + 0.152 = 2.21% of protein.

MP 6. Amino Acids Estimation

Amino acid composition is important for determining the nutritive value of plant proteins. Ion exchange chromatography is commonly used to estimate the amino acid content of samples using an amino acid analyzer. The grain proteins are converted to free amino acids by hydrolysis with 6 N HCl. The mixture of amino acids in the protein hydrolyzate is passed through a column of sulfonated polystyrene resin where the individual amino acids are separated. Each amino acid reacts with ninhydrin and the absorbance of the color produced is recorded. The concentration of amino acid is determined by using an integrator (Moore and Stein 1963).

Equipment

Balance, flash evaporator, refluxing unit, pH meter, Beckman 119CL amino acid analyzer, HP 3390A integrator, magnetic stirrer, and rotary evaporator.

Reagents

a. Hydrochloric acid, 6 N. Take 500 mL of distilled water in a 1000 mL beaker. Add 500 mL concentrated hydrochloric acid and mix well.

b. Sodium citrate buffer, pH 2.2 (0.2 N). Dissolve 19.6 g of sodium citrate ($Na_3C_6H_2O_7$ 2 H_2O) in 800 mL of distilled water. Mix 16.5 mL of concentrated hydrochloric acid (HC1) and 5 mL of thiodioglycol. Stir the mixture using a magnetic stirrer until dissolved. Adjust the pH to 2.2 and add 0.1 mL of octanoic acid. Make up the volume to 1000 mL with distilled water. (This buffer is used for dissolving the protein hydrolysate for amino acid analysis).

c. Sodium citrate buffer, pH 3.25 (0.2 N sodium) (Buffer A). Dissolve 78.4 g of sodium citrate in 3.5 L of distilled water. Mix 50.3 mL of cone. HCl and 10 mL of thiodioglycol. Stir the mixture with a magnetic stirrer until dissolved. Adjust the pH to 3.25. Add 0.4 mL of octanoic acid and make up the volume to 4000 mL with distilled water.

d. Sodium citrate buffer, pH 3.95 (0.4 N sodium) (Buffer B). Dissolve 78.4 g of sodium citrate in 3.5 L of distilled water. Mix 46.8 g of sodium chloride, 35.2 mL of conc. HCl and 10 mL of thiodioglycol. Stir the mixture in a magnetic stirrer. Adjust the pH to 3.95. Add 0.4 mL of octanoic acid and make up the volume to 4000 mL with distilled water.

e. Sodium citrate buffer pH 6.40 (1.0 N sodium) (Buffer C). Dissolve 78.4 g of sodium citrate in 3.5 L of distilled water. Mix 187.2 g of sodium chloride, and 1.0 mL of conc. HCl. Stir the mixture in a magnetic stirrer. Adjust the pH to 6.40. Add 0.4 mL of octanoic acid. Make up the volume to 4000 mL with distilled water.

f. Sodium citrate buffer, pH 5.5 (0.35 N sodium for lysine). Dissolve 137.3 g of sodium citrate in 3.5 L of distilled water and mix well by using a magnetic stirrer. Add 26.2 mL of conc. HCl. Adjust the pH to 5.26. Add 0.4 mL of octanoic acid. Make up the volume to 4000 mL with distilled water.

g. Sodium citrate buffer, pH 5.5 (0.158 N sodium for tryptophan). Dissolve 61.98 g of sodium citrate in 3.5 L of distilled water and mix well using a magnetic stirrer. Add 11.8 mL of conc. HCl. Adjust the pH to 5.26. Add 0.4 mL of octanoic Acid. Make up the volume to 4000 mL with distilled water.

h. **Sodium hydroxide, 0.2 N {Regeneration solvent).** Dissolve 8 g of sodium hydroxide (NaOH) in distilled water. Make up the volume to 1000 mL with distilled water.

i. **Sodium acetate buffer (for ninhydrin reagent preparation).** Dissolve 2.720 g of sodium acetate and mix in 2.5 L of distilled water using a magnetic stirrer. Add 500 mL of glacial acetic acid. Adjust the pH to 5.5. Make up the volume to 5000 mL with distilled water. Filter the above reagent through a 0.4-0.6 µm filter.

j. **Ninhydrin reagent preparation.** Mix 2.85 L of methyl cellosolve and 950 mL of sodium acetate buffer pH 5.5 (Buffer C, Reagent d) using a magnetic stirrer. Purge with nitrogen gas for 15 min through the solution. Add 76 g ninhydrin using a funnel. Continue purging with nitrogen until the ninhydrin is completely dissolved. While the ninhydrin is dissolving add 1 ampoule (7.5 mL) of titanous chloride (Pierce 27750). Connect the ninhydrin line on to the amino acid analyzer along with nitrogen, with a pressure maintained at 0.21-0.28 kg cm^{-2}.

Note: When all the above buffers are prepared they are transferred to their respective labeled bottles (labeled as Buffer A, B, C, etc.,) and connect to the respective ports in che amino acid analyzer.

Preparation of amino acid standards. Add 5 mL of commercially available amino acid standard solution (either from Sigma, Beckman, or Pierce companies) to 5 mL for internal standards containing 2.5 µmol mL^{-1} of each compound (i.e., cysteic acid, methionine sulfoxide, methionine sulfone, and norleucine) and make up to 25 mL with 0.2 N sodium citrate buffer (pH 2.2).

Internal amino acid standard preparation. Dissolve 46.8 mg of cysteic acid, 41.4 mg methionine sulfoxide, 45.3 mg methionine sulfone, and 32.795 mg norleucine in 0.2 N sodium citrate buffer (pH 2.2) and make up to 100 mL. This gives a concentration of 2.5 µmole mL^{-1} of each of the above amino acids.

Sample preparation

a. Weigh 50 mg of a defatted sample into a 150 mL flat bottom flask and add 50 mL of 6 N HCl and a few boiling chips.

b. Reflux in a refluxing unit for 24 h, cool and flash-evaporate the acid using a rotary evaporator. Repeat the procedure by giving three washings with water and dry it completely.

c. Dissolve the contents of the flask in a small quantity of 0.2 N sodium citrate buffer, pH 2.2 and add 0.5 mL of 5 µmole mL^{-1} norleucine (internal standard) and make up to 4.5 mL with the above buffer.

d. Filter the aliquot through a Whatman No 41 filter paper or Udy filter and use 0.1 mL for the analysis in the amino acid analyzer.

Setting the 119 CL Beckman Amino Acid Analyzer

- Set the control module switch (main power) 'on'.
- Set the A & B buffer-change controls at 27 min and in 'Auto' position.
- Set the A & B buffer-change 2 A or B/C control at 44 min and in 'Auto' position.
- Set the regeneration control at 75 min and in 'Auto' position.
- Set the master control at 77 min and in 'Auto' position.

- Set the ninhydrin valve control at 20 min and in 'Auto' position.

When run on 'manual' change all the control knobs to manual position.

Setting the pump switches

- First switch 'On' the buffer pump and look for the required flow rate (44 mL h^{-1}.)
- Switch 'On' the ninhydrin pump and look for the required flow rate (22 mL h^{-1}) .

The combined flow rate should be 66 mL h^{-1}. This can be measured by using the bubble flow indicator on the front panel of the instrument.

Setting the colorimeter controls

- Adjust the base line by two duodials one for 440 nm and the other for 57 0 nm.
- **Wavelength.** A rotary switch for 440 nm and 570 nm is available on the front panel of the instrument. When run on 'Auto' the sampler uses the 'Sum' of the rotary switches. Adjust the full-scale range for that channel to 2.0 A (absorbance).
- **Integrator range.** Position the integrator range to 2.0 A. Regardless of the absorption during the run this switch will work on the integrator position.
- **Temperature control.** Temperature of the resin column should be on two ranges (50°C and 65°C).
- **Recorder speed.** Adjust to 152.4 mm h^{-1}.

Loading the amino acid standard or hydrolyzed sample through the auto sampler

When all the above settings on the Beckman 119 CL amino acid analyzer are ready, load 100 µL amino acid standard or hydrolyzed sample through the auto sampler. To load, fill the auto sample loop with sodium citrate buffer (pH 2.2) **(Reagent** g), using a syringe. Place a 100 µL micropipette containing the hydrolyzate into the sample loop and suck the other side of the loop using a syringe. Insert an air bubble between the sample and buffer. Then move to the next sample by using the switch provided on the auto sampler. Load the series of samples as required (up to 30 samples can be loaded).

Injection of sample

Using the injection toggle switch, inject the sample. Since the instrument is already running, the pumps go 'off, for a few minutes. This is followed by the automatic 'start' of the pumps and the elution starts. Simultaneously the integrator starts working.

Calculation

Run the standards along with the samples (0.5 µmoles mL^{-1}). In the case of the protein hydrolyzate, the amino acids are eluted in the following order: Cysteic acid, methionine sulfoxide, aspartic acid, methionine sulfone, threonine, serine, glutamic acid, proline, glycine, alanine, half cystine, valine, methionine, isoleucine, leucine, norleucine, tyrosine, phenylalanine,

histidine, lysine, ammonia, and arginine.

Each amino acid is calculated as given below (an example of calculating the concentration of lysine is given) .

Mass of sample	50 mg
Sample volume	5 mL
Protein in the sample	9.7%
Peak area of sample (Lys)	0.45
Peak area of standard (Lys)	1.31
Conc. of standard (Lys)	50 nanomoles = 0.05 µmoles

$$\text{Concentration of sample (Lys)} = \frac{0.4\ 5 \times 50\ \text{nm}}{1.31} = 17.18\ \text{nanomoles}$$

Molecular weight (MW) of Lysine = 146.19

$$\text{Concentration of Lysine (µg)} = \frac{17.18\ \text{nm} \times 146.19\ (\text{MW})}{1000} = 2.512\ \mu\text{g}$$

$$\text{Lysine g (100 g)}^{-1}\ \text{sample} = \frac{2.512\ (\mu\text{g}) \times 5\ (\text{mL}) \times 100\ (\%)}{0.05 \times 0.1\ (\text{mL}) \times 1000 \times 1000}\ 0.2512\ (\%)$$

$$\text{Lysine g (100 g)}^{-1}\ \text{protein} = \frac{0.2512 \times 100\ (\%)}{9.7\ (\%)} = 2.59\ (\%)$$

Where, 9.7% is protein content in the sample.

In a similar way, the concentrations of other amino acids are calculated.

MP 7. Methionine and Cystine Estimations

The estimation of methionine and cystine is initiated by converting them to methionine sulfone and cysteic acid with performic acid. Their concentrations are then determined by using an amino acid analyzer (Speckman et al. 1958; Moore 1963).

Equipment

Balance, vacuum desiccator, vacuum pump, reflux units, flash evaporator, pH meter, 119 CL amino acid analyzer, and magnetic stirrer.

Reagents

a. **Performic acid reagent.** Add 1 mL of 30% H_2O_2 to 9 mL of 83% formic acid. Let the mixture stand for 1 h at room temperature and then cool to 0°C.

b. **Hydrogen bromide (48%) solution.**

c. **NaOH pellets.**

d. **Standard cysteic acid and methionine sulfone solutions (0.5 µm mL^{-1}).**

e. **6 N HCl.**

f. **0.2 N sodium citrate buffer, pH 2.2** (Refer section MP 6).

Procedure

1. Weigh a sample containing 2-5 mg protein in a 150 mL flask.
2. Add 2 mL of **reagent a** to the flask. Let the mixture stand in the refrigerator overnight.
3. Add 0.3 mL of hydrogen bromide solution **(Reagant b)** while swirling the flask in an ice bath.
4. Place the flask in a vacuum desiccator over sodium hydroxide pellets. Apply a partial vacuum until the bromine distills over and then apply a full vacuum to evaporate the solution to dryness.
5. Hydrolyze the sample with 6 N HCl at 110°C for 24 h in an oven.
6. Cool and flash-evaporate the hydrochloric acid using a rotary evaporator.
7. Dissolve the solid residue in 5 mL of sodium citrate buffer, filter through a udy filter and use 0.1 mL of the solution for analysis in the amino acid analyzer.
8. Follow the procedure that is given in section MP 6.
9. Run cysteic acid and methionine sulfone standards for comparison.
10. Calculate the concentration of methionine and cystine as given for other amino acids in section MP 6.

MP 8. Tryptophan Estimation

Tryptophan is released from the protein in the sample by hydrolyzing with alkali. The tryptophan thus produced is analyzed using an amino acid analyzer (Hugli and Moore 1972) .

Equipment

Balance, oven, pH meter, centrifuge, 119 CL Beckman amino acid analyzer, and magnetic stirrer.

Reagents

a. 5 N NaOH. Dissolve 50 g sodium hydroxide pellets in distilled deionized water and make up to 250 mL.

b. 0.2 N sodium citrate buffer, pH 4.25. Refer section MP 6.

c. Tryptophan standard. Dissolve 25 mg L-Tryptophan in sodium citrate buffer and make up to 100 mL (stock solution). From the stock solution dilute 1 mL to 10 mL with sodium citrate buffer (which will give 25 µg of tryptophan mL^{-1}).

Procedure

1. Weigh 100-200 mg of a defatted sample into a polypropylene centrifuge tube, add 2.5 mL 5N NaOH (Reagent a) and place the centrifuge tube inside a glass test tube and cover it with a stopper. Seal the test tube under a vacuum.

2. Hydrolyze the contents at 110°C for 24 h. After hydrolyzing transfer the contents into a beaker and neutralize the alkali with acid to pH 4.25 in an ice bath.

3. Make the volume up to 10 mL with sodium citrate buffer.

4. Centrifuge the solution if it is cloudy.

5. Use 0.1 mL of the solution for analysis in a 119 CL Beckman amino acid analyzer. Follow the procedure given in section MP 6.

Run a tryptophan standard and then calculate percentage tryptophan as shown for other amino acids in section MP 6.

MP 9. Starch Estimation

Starch is converted into maltose and finally glucose on hydrolysis with the amyloglucosidase enzyme. The sugar thus obtained is analyzed quantitatively using the phenol-sulfuric acid reagent (Dubois et al. 1956; Southgate 1976).

Equipment

Balance, autoclave, water bath, pH meter, and spectrophotometer.

Reagents

a. Ethanol.

b. 2 M sodium acetate buffer (pH 4.8). Dissolve 164 g sodium acetate in distilled water and add 120 mL glacial acetic acid, adjust the pH to 4.8 and make up to 1 L with distilled water.

c. Amyloglucosidase (Sigma).

d. 5% phenol. Dissolve 5 g phenol in distilled water and make up to 100 mL with distilled water.

e. 96% sulfuric acid (v/v). Use 98% sulfuric acid, specific gravity 1.84, and dilute according to the purity.

f. Glucose (w/v) standard [Stock = 1000 mg (1000 mL)$^{-1}$]. Dissolve 1000 mg glucose in distilled water and make up to 1 L.

g. Working standard. Pipette out 10 mL of stock standard into a 100 mL volumetric flask and make up volume to 100 mL (the final concentration will be 100 µg mL^{-1}).

Procedure

1. Weigh 75 mg of a defatted cereal flour in to a 50 mL conical flask.
2. Add a few drops of ethanol to disperse the flour.
3. Add 10 mL of distilled water.
4. Cover the flask with nitrogen-free paper.
5. Autoclave the contents for 90 min at a pressure of 1.34 kg cm^{-2} (126°C) to gelatinize the starch.
6. Cool and add 1 mL 2 M sodium acetate buffer (Reagent b).
7. Add 25 mg amyloglucosidase and approximately 15 mL distilled water.
8. Cover the contents with parafilm and incubate at 55°C for 2 h on a water-bath shaker (hydrolysis).
9. After incubation make up the volume to 250 mL with distilled water.
10. Pipette out a 10 mL aliquot and dilute to 100 mL with distilled water.
11. Pipette out 1 mL of the above aliquot and add to 1 mL 5% phenol (Reagent d) and 5 mL 96% sulfuric acid (Reagent e) and mix well.

12. Run glucose standards with different concentrations (i.e., 10, 20, 30, 40, and 50 µg of glucose) from the working standard, keeping the volume to 1 mL with distilled water; add reagents as in step 11.

13. Run a blank containing enzyme, buffer, and reagent (water, phenol, and sulfuric acid).

14. Cool and read the absorbance at 490 nm against the blank.

Calculation

$$\frac{\text{Conc. of std. (µg) x 250 mL (sample dilution) x 100 (percent) x 0.9}}{\text{Absorbance of standard x 1 000 000 x 0.1 mL (taken for color development) x 0.075 g (sample mass)}}$$

0.9 = Conversion factor for starch into glucose.

Starch (%) = Factor x Absorbance for sample aliquot (1 mL).

Example

Concentration of glucose standard = 20 µg mL^{-1}
Absorbance of standard = 0.2 optical density (A)
Absorbance for the sample aliquot = 0.232
Sample mass = 0.075 g

$$\text{Distribution factor} = \frac{20\ \mu g\ mL^{-1} \times 250\ mL \times 0.9 \times 100}{0.2\ (A) \times 1\ 000\ 000 \times 0.1\ mL \times 0.075\ g} = 300$$

Starch (%) = Factor x A (Absorbance for the sample aliquot)

Starch (%) = 300 x 0.232 = 69.6%

MP 10. Soluble Sugars Estimation

Soluble sugars are extracted with hot aqueous ethanol. The sugars on treatment with phenol-sulfuric acid produce a stable and sensitive golden-yellow color. This method can be applied to simple sugars, oligosaccharides, polysaccharides, and their derivatives (Dubois et al. 1956).

Equipment

Balance, Vortex mixer, hot plate, boiling tubes, filter papers, sand bath, water bath, and spectrophotometer.

Reagents

a. **80% ethanol**. Mix 800 mL of ethanol in distilled water and make up to 1 L with distilled water.

b. **5% phenol**. Dissolve 5 g phenol in distilled water and make up to 100 mL with distilled water.

c. **96% sulfuric acid (v/v)**. Use 98% sulfuric acid (specific gravity 1.84) and dilute according to the purity.

d. **Glucose standard (w/v)**: Dissolve 1 g glucose in water and make up to 1 L.

e. **Working standard**. Pipette out 10 mL of glucose standard (**Reagent d)** to a 100 mL volumetric flask and make up to 100 mL (the final concentration will be 100 µg mL^{-1}) .

Procedure

1. Weigh 100 mg defatted flour into a boiling tube.
2. Add 25-30 mL of hot 80% ethanol (Reagent a) into the boiling tube and shake on a Vortex mixer.
3. Let the material in the tube settle for 20 min.
4. Filter the extract into a beaker through a Whatman No. 41 filter paper.
5. Repeat steps 2 to 4, three or four times to ensure complete extraction of sugars.
6. Heat the extract on a hot-sand bath until the ethanol is evaporated. Do not over heat to prevent caramalization.
7. Dissolve the contents with a small quantity of distilled water and transfer into a 100 mL volumetric flask. Make up to 100 mL with distilled water.
8. Pipette 1 mL aliquot from the above and prepare a 1 mL water blank.
9. Add 1 mL 5% phenol **(Reagent b)** to the sample and water blank and shake.
10. Add 5 mL 96% sulfuric acid **(Reagent a)** to each and shake vigorously on a Vortex mixer; cool the tubes in a water bath.
11. Read absorbance of the golden yellow color of the solution at 490 nm against the blank.

12. Pipette 0.2 mL working standard glucose into a test tube and add 0.8 mL distilled water, and follow the steps 9 to 10.

Calculation

Total soluble sugars (%) = Factor x Absorbance of sample

$$\text{Factor} = \frac{\text{Conc. of std. } (\mu g\ mL^{-1}) \times 100\ mL\ (\text{volume of extract made}) \times 100}{\text{Absorbance of std.} \times 1\ 000\ 000 \times \text{Sample aliquot } (1\ mL) \times g\ (\text{sample mass})}$$

Example

Concentration of the standard = 20 µg mL^{-1}
Absorbance for the standard = 0.20
Sample mass = 0.10 g
Sample aliquot = 1 ml
Absorbance for the sample aliquot = 0.25

$$\text{Factor} = \frac{20\ \mu g\ mL^{-1} \times 100\ mL \times 100\ g}{0.2 \times 1\ 000\ 000 \times 1\ mL \times 0.1\ g} = 10$$

Soluble sugars (%) = Factor x Absorbance for the sample aliquot.

= 10 x 0.25% = 2.5%

MP 11. Ash Estimation

The sample is ignited to burn off all organic matter in the sample. The inorganic material which does not volatilize at that temperature is ash that is gravimetrically determined (Association of Official Analytical Chemists 1984).

Equipment

Balance, muffle furnace, porcelain crucibles, and desiccator.

Procedure

1. Set the temperature of the muffle furnace at 600°C and place clean crucibles in the muffle furnace for 1 h. Transfer them into a desiccator and cool them to room temperature, then weigh (W_1) each crucible.

2. Place about 2 g of a moisture-free cereal sample into a weighed crucible and weigh (W_2) .

3. Incinerate the sample at 600°C for about 2 h.

4. Transfer the crucibles into a desiccator and cool to room temperature and then weigh (W_3). The mass should be taken as quickly as possible to prevent moisture absorption.

5. Repeat the incineration until a constant mass is obtained.

Calculation

$$\text{Ash (\%)} = \frac{\text{Mass of ash} \times 100}{\text{Mass of sample}} = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1)}$$

Example

W_1 = Mass of crucible = 5.0654 g
W_2 = Mass of crucible + sample = 7.0785 g
W_3 = Mass of crucible + ash = 5.1020 g

$$\text{Ash (\%)} = \frac{(5.1020\ \text{g} - 5.0654\ \text{g}) \times 100}{7.0785\ \text{g} - 5.0654\ \text{g}} = 1.82\%$$

MP 12. Tannin Estimation

The vanillin-hydrochloric acid procedure for tannin estimation is based on the formation of a color complex that is absorbed at 500 nm and is generally called the catechin equivalent (CE). This reaction is specific for leucoanthocyanidins, although authocyanins and dihydrochalcones may interfere to some extent (Price et al. 1978).

Equipment

Balance, centrifuge, centrifuge tubes, shaker, and spectrophotometer.

Reagents

a. **8% hydrochloric acid in methanol.** Mix 8 mL concentrated HC1 in methanol and make up to 100 mL with methanol.

b. **Vanillin.** Dissolve 1 g vanillin (Sigma) in methanol and make up to 100 mL with methanol.

c. **Vanillin-hydrochloric acid reagent.** Mix equal volumes of solutions 'a' and 'b' before use.

d. **4% hydrochloric acid in methanol.** Mix 4 mL concentrated HCl with 96 mL methanol.

e. **1% hydrochloric acid in methanol.** Mix 1 mL concentrated HCl with 99 mL methanol.

f. **Standard solutions.** Prepare a stock solution, 1 mg mL^{-1} of catechin (Sigma) in methanol. The stock solution can be stored for long periods in stoppered bottles under cool conditions. A 0.1 mL of stock solution is equivalent to 100 µg catechin mL^{-1}. Pipette out 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of catechin standard into separate tubes, and make up each to 1 mL with methanol.

Procedure

Extraction

1. Weigh 500 mg defatted-flour sample and transfer it to a centrifuge tube.
2. Add 10 mL of acidic-methanol **(Reagent e)** into each tube and shake for 20 min on a shaker.
3. Centrifuge for 10 min and transfer the extract to a 20 mL volumetric flask.
4. Add 5 mL of acidic-methanol **(Reagent e)** to the centrifuge tube and shake for 20 min.
5. Centrifuge for 10 min and transfer the extract to the first extraction. Make up the volume to 20 mL (with **reagent e**) and mix well.

Estimation

1. Pipette 1 mL of the extract into a test tube.

2. Add 5 mL freshly prepared vanillin-HCl (Reagent c) slowly to the extract and to the catechin standards of different concentrations (standard solutions).

3. Prepare individual sample blanks by adding 5 mL of 4% HCl in methanol (Reagent d) to 1 mL distilled water. (Blanks will be without vanillin-HCl reagent).

4. Read the absorbance at 500 nm against the sample blank.

Calculation

Prepare a standard curve by plotting the average absorbance readings of the duplicate determinations of the standard catechin concentrations. Use one concentration of the linear standard for calculation (e.g., 1 mg mL^{-1}).

Subtract the blank absorbance (absorbance) from the sample absorbance. Calculate the catechin equivalents (CE%) as follows:

$$\text{Catechin equivalents (\%) in sample} = \frac{\text{Absorbance for 1 mL sample aliquot} \times \text{Volume made-up}}{\text{Absorbance for 1 mL catechin standard (1 mg mL}^{-1}\text{)}}$$

$$\times \frac{1 \text{ mg} \times 100}{\text{Volume taken for estimation} \times \text{sample mass (mg)}}$$

Example

Absorbance of the sample = 0.295
Absorbance of the blank = 0.012
Difference (0.295 - 0.012) = 0.283
Made-up volume = 20 mL
Volume of aliquot taken for analysis = 1 mL
Sample mass = 500 mg

Volume of the catechin standard = 1 mL
Catechin present in 1 mL = 1 mg
Absorbance for 1 mL catechin = 0.5

$$\text{CE (\%)} = \frac{0.283 \times 20 \text{ mL} \times 100}{0.5 \times 1 \text{ mL} \times 500 \text{ mg}} = 2.264\%$$

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Evaluation

Select the most appropriate answer and check the correct answer at the end of the booklet.

MP 1. Breaking Dormancy in Pearl Millet

1. The inability of the physiologically matured seed to germinate under favorable conditions is
   a) immaturity. b) incompatibility.
   c) precocity. d) dormancy.

2. The percentage of KNO_3 that was used to break the dormancy in pearl millet was
   a) 2.2. b) 2.0. c) 0.2. d) 22.0.

3. The period for which the pearl millet seed was incubated after the KNO_3 treatment, to break the dormancy in pearl millet was
   a) 18 days. b) 28 days, c) 20 days. d) 8 days.

4. The temperatures which were alternately followed for incubation after the KNO_3 treatment for breaking dormancy in pearl millet were
   a) 1°C and 10°C. b) 20°C and 40°C.
   c) 30°C and 60°C. d) 5°C and 35°C.

5. The two chemicals that were suggested by Burton (1969) for breaking the dormancy in pearl millet were
   a) potassium hydroxide and chloroform.
   b) sodium hydroxide and potassium chloride.
   c) 2-chloroethanol and potassium hypochlorite.
   d) benzine hexachloride and potassium permanganate.

6. The period to which the treatment with the two chemicals suggested by Burton (1969) for breaking the dormancy in pearl millet was
   a) 1 h. b) 5 h. c) 10 h. d) 24 h.

7. The temperature at which the water-soaked seed was incubated to break dormancy in pearl millet was
   a) 5°C. b) 20°C. c) 50°C. d) 35°C.

8. The period for which the water-soaked seed was incubated to break the dormancy in pearl millet was
   a) 4 h. b) 24 h. c) 20 h. d) 40 h.

9. The chemical that was used for treating the pearl millet seed to break the dormancy was
   a) ethephon. b) ethanol.
   c) ethylamine. d) ethylene.

MP 2. Moisture Estimation

1. A _____ sample was used for moisture determination.
   a) 10.0 g b) 6.0 g c) 2.5 g d) 1.0 g

2. The drying of the sample for moisture determination was done in a/an
   a) oven. b) muffle furnace.
   c) desiccator. d) thermostat.

3. The sample was dried at 100°C for
   a) 5 h. b) 1 h. c) 12 h. d) 30 min.

4. The sample was dried for 2 h at
   a) 130°C. b) 100°C. c) 50°C. d) 30°C.

5. The dried sample was cooled in a/an
   a) reflux unit. b) flash evaporator.
   c) desiccator. d) oven.

6. The percentage of moisture in the sample with the following data is
   Mass of aluminum dish .. .. .. 25.2121 g
   Mass of dish + flour sample .. .. .. 27.5625 g
   Mass of dish + dried and cooled sample .. 25.3656 g

   a) 5.2 b) 1.0 c) 11.2 d) 10.0

MP 3. Oil Estimation

1. The _____ was used to extract oil.
   a) block digester b) Technicon auto analyzer
   c) Soxhlet apparatus d) flask evaporator

2. The oil was extracted by using a
   a) solvent. b) vacuum pump,
   c) catalyst. d) desiccator.

3. A _____ sample was used for oil estimation using a Soxhlet apparatus with 200 mL capacity flask.
   a) 40 g b) 10 g c) 14 g d) 4 g

4. _____ was used in the oil estimation.
   a) Xylol b) Ethylene,
   c) Ethanol. d) Hexane.

5. The extraction of oil was usually done for
   a) 10 h. b) 18 h. c) 8 h. d) 28 h.

6. The solvent was evaporated by using a
   a) sand bath. b) vacuum pump,
   c) centrifuge. d) vortex mixer.

7. The last traces of solvent were removed in a/an
   a) centrifuge. b) refluxing unit.
   c) desiccator. d) oven.

8. The percentage of oil in the sample with the following data is
   Mass of the beaker + oil .. .. 95.235 g
   Mass of the beaker .. .. .. 95.121 g
   Mass of the sample .. .. .. 4.125 g

   a) 2.76 b) 27.6 c) 72.2 d) 7.62

MP 4. Protein Estimation

1. The two chemicals that were mixed to prepare the catalyst were
   a) ferric chloride and potassium sulfide.
   b) potassium chloride and mercuric chloride.
   c) potassium sulfate and mercuric oxide.
   d) calcium sulfate and ferrous sulfate.

2. The two chemicals that were mixed to prepare 60% sodium hydroxide for protein estimation were
   a) sodium hydroxide and sodium chloride.
   b) sodium bicarbonate and sodium hydroxide.
   c) sodium carbonate and sodium thiosulfate.
   d) sodium hydroxide and sodium thiosulfate.

3. The proportion of methyl red (MR) and bromocresol green (BCG) that were mixed to prepare the double indicator were
   a) 1 MR and 1 BCG. b) 5 MR and 1 BCG.
   c) 1 MR and 2 BCG. d) 5 MR and 3 BCG.

4. The normality of hydrochloric acid that was used in the estimation of protein was
   a) 0.2 N. b) 2.0 N. c) 0.02 N. d) 2.2 N.

5. The quantity of ground sorghum grain sample that is usually taken for digestion in the protein estimation by the microkjeldahl procedure is
   a) 10 mg. b) 100 mg. c) 20 mg. d) 50 mg.

6. The digestion flask that was used in the protein estimation was a
   a) microdosimeter. b) microlite.
   c) microtome. d) Microkjeldahl.

7. The quantity of digestion mixture that was added to the sample in protein estimation by the Mkj method was
   a) 100 g. b) 10 g. c) 20 g. d) 2 g.

8. The quantity of sulfuric acid dispensed to the digestion flask in the protein estimation using microkjeldahl procedure was
   a) 2 mL. b) 30 mL. c) 40 mL. d) 20 mL.

9. The digestion period of the sample in the protein estimation was
   a) 30 min. b) 1 h. c) 3 h. d) 10 min.

10. The quantity of sodium hydroxide solution added before the distillation in the microkjeldahl procedure was
    a) 50 mL. b) 1 mL. c) 10 mL. d) 100 mL.

11. The quantity of boric acid required to collect the distillate in the microkjeldahl procedure was
    a) 25 mL. b) 55 mL. c) 15 mL. d) 5 mL.

12. The distillate was titrated against
    a) boric acid. b) hydrochloric acid.
    c) lactic acid. d) nitric acid.

13. The equivalent mass of nitrogen is
    a) 0.1407. b) 1.4017. c) 14.007. d) 7.014.

14. The factor used to convert cereal N % to protein is
    a) 16.25. b) 14.25. c) 5.25 d) 6.25

15. The protein percentage of a pearl millet grain sample with the following data from the Microkjeldahl method was

Normality of acid	0.02 N
Titer value (TV)	5.72 mL
Mass of the sample	76.20 mg
Equivalent weight of nitrogen	14.0007

    a) 12.1. b) 3.1. c) 23.1. d) 13.1.

16. The two acids that were mixed for using in the protein estimation with the Technicon Auto Analyzer were
    a) sulfuric acid and hydrochloric acid.
    b) boric acid and sulfuric acid.
    c) hydrochloric acid and orthophosporic acid.
    d) orthophosphoric acid and sulfuric acid.

17. The percentage of sodium hypochlorite used in the Technicon auto analyzer protein estimation was
    a) 20. b) 10. c) 5. d) 25.

18. The normal quantity of pearl millet flour sample required for Technicon auto analyzer protein estimation was
    a) 1 mg. b) 10 mg. c) 100 mg. d) 20 mg.

19. The temperature maintained in the block digester for protein estimation by TAA method was
    a) 37°C. b) 375°C. c) 305°C. d) 75°C.

20. The protein percentage of a pearl millet grain sample with the following data from the Technicon auto analyzer was

Sample peak height (Net division)	45
Sample mass	100 mg
Net divisions on the chart paper for 10 ppm N.	1.75
Made-up volume	75 mL

a) 10.05. b) 22.05 c) 2.05. d) 12.05.

21. The dye that was used in the "Dye binding capacity method" was
a) methyl red. b) acid orange 12.
c) bromocresol green. d) prussian blue.

22. The percentage of protein was determined as the amount of dye _____ the basic amino acids found in the protein.
a) that was present in b) that dissolved
c) that bound to d) that can synthesize

23. The correlation (r) value between the "Dye-binding-capacity method" and the 'Microkjeldahl' method of protein estimation was
a) 0.9. b) 0.5. c) 0.2. d) 0.1.

24. The concentration of the reagent dye used in the DBC method of protein estimation was
a) 13 mg mL^{-1} b) 1.3 mg mL^{-1}
c) 2.3 mg mL^{-1} d) 3.2 mg mL^{-1}

25. The concentration of reference dye used in the DBC method was
a) 6 mg mL^{-1} b) 3.6 mg mL^{-1}
c) 6 mg mL^{-1} d) 0.6 mg mL^{-1}

26. The quantity of pearl millet flour used in the DBC method was
a) 10 g. b) 20 g. c) 1 g. d) 100 g.

27. The quantity of reagent dye added to the flour sample in DBC method was
a) 4 mL. b) 10 mL. c) 100 mL. d) 40 mL.

28. The formula used for the estimation of protein in the DBC method is
a) m (UIR) + C. b) m + UIR + C.
c) m (UIR) - C. d) m + (UIR) C.

29. The factor "m" used in the formula for the estimated Microkjeldahl protein percentage is the
a) regression coefficient. b) correlation value.
c) covariance. d) coefficient of variation.

30. The factor "C" used in the formula for the estimated Microkjeldahl protein percentage refers to the
a) degrees of freedom.
b) critical difference.
c) intercept from the regression equation.
d) standard deviation.

31. The estimated Microkjeldahl protein percentage in an experiment with the following data is: m = 0.298, UIR = 32.8%, C = 1.215

a) 0.98%. b) 10.98%. c) 1.98%. d) 11.98%.

MP 5. Lysine Estimation

1. The amount of sample usually taken for lysine estimation is
a) 10 g. b) 20 g. c) 1 g. d) 5 g.

2. The dye used in the lysine estimation is
   a) methylene blue. b) acid orange.
   c) phenolphthalein. d) methyl orange.

3. The quantity of dye solution used in lysine estimation is
   a) 40 mL. b) 10 mL. c) 5 mL. d) 1 mL.

4. The equipment used for the estimation of lysine percentage is the
   a) Mkj distillation set. b) reciprocating shaker,
   c) filter set. d) Udy instrument.

5. The estimated lysine percentage is obtained by the ratio of
   a) UIR/N. b) UIR/P.
   c) P/UIR. d) N/UIR.

MP 6. Amino Acids Estimation

1. Food grain proteins are converted to amino acids by
   a) dehydration. b) osmosis,
   c) hydrolysis. d) polarization.

2. _____ was used in hydrolysis to convert protein into amino acids.
   a) KNO_3 b) $NH_3(SO_4)2$ c) 6NHC1 d) H_2SO_4

3. A _____ solution of NaOH was obtained by dissolving 8 g of NaOH in 1 L of water.
   a) 2.2 N b) 2.0 N c) 0.2 N d) 1.2 N

4. The quantity of defatted sample was used for amino acid analysis is
   a) 150 mg. b) 500 mg. c) 0 mg. d) 5 mg.

5. The quantity of 6 N HC1 that was added to the sample for hydrolysis for estimation of amino acids was
   a) 500 mL. b) 100 mL. c) 50 mL. d) 250 mL.

6. The sample mixture was refluxed for _____ with the chemical.
   a) 48 h b) 2 h c) 10 h d) 24 h

MP 7. Methionine and Cystine Estimations

1. The methionine is estimated as
   a) methyl alcohol. b) methane.
   c) methionine sulfone. d) methylamine.

2. Cystine was converted to _____ for its estimation,
   a) carbonic acid b) acetic acid
   c) citric acid d) cysteic acid

3. Performic acid is prepared by mixing
   a) H_2O and formaldehyde. b) H_2O_2 and formic acid.
   c) H_2O and ferric acid. d) H_2O_2 and fatty acid.

4. For the estimation of methionine use _____% HBr.
   a) 48 b) 28 c) 38 d) 18

5. Sodium citrate used in the estimation of methionine is a _____ solution.
   a) 1.2 N b) 2.2 N c) 2.0 N d) 0.2 N

6. The quantity of HBr used in methionine estimation was
   a) 3 mL. b) 13 mL. c) 30 mL. d) 10 mL.

7. The sample was hydrolyzed for 24 h for amino acids estimation at
   a) 100°C. b) 50°C. c) 110°C. d) 210°C.

8. The reagent used to dissolve the amino acids was
   a) sodium citrate. b) potassium chloride.
   c) calcium sulfate. d) sodium hydroxide.

9. The quantity of the aliquot used for analysis in the amino acid analyzer was
   a) 1.0 mL. b) 5.0 mL. c) 2.0 mL. d) 0.1 mL.

10. The instrument used for analyzing the amino acids was a/an
   a) flask evaporator. b) amino acid analyzer,
   c) vacuum desiccator. d) pH meter.

MP 8. Tryptophan Estimation

1. A 5N sodium hydroxide solution is prepared by mixing
   a) 100 g NaOH in 150 mL water.
   b) 50 g NaOH in 250 mL water.
   c) 5 g NaOH in 100 mL water.
   d) 0.5 g NaOH in 50 mL water.

2. A _____ sodium citrate was used in the tryptohan analysis.
   a) 2.0 N b) 2.2 N c) 4.0 N d) 0.2 N

3. The quantity of defatted sample used for tryptophan estimation was
   a) 800 mg. b) 5 mg. c) 1300 mg. d) 150 mg.

4. The sample was weighed in to a _____ tube.
   a) polypropylene b) paraldehyde
   c) phenolphthalein d) polyethylene

5. The quantity of NaOH solution added to the sample for tryptophan estimation was
   a) 1.0 mL. b) 2.5 mL. c) 25 mL. d) 4.5 mL.

6. The sample mixture was hydrolyzed for tryptophan estimation at
   a) 50°C. b) 200°C. c) 110°C. d) 150°C.

7. The sample was hydrolyzed for tryptophan estimation for
   a) 5 h. b) 1 h. c) 10 h. d) 24 h.

8. An aliquot of _____ was used for tryptophan analysis using an amino acid analyzer.
   a) 1.0 mL b) 0.1 mL c) 2.0 mL d) 2.2 mL

9. The type of amino acid analyzer that was used for tryptophan analysis was
   a) the 119 CL Beckman. b) the Technicon auto analyzer.
   c) the Kjeldahl. d) a chromatograph.

MP 9. Starch Estimation

1. The components of starch are
   a) albumen and acrylic acid. b) amylose and pectin.
   c) amylose and amylopectin. d) acetic acid and adrenalin.

2. The enzyme that hydrolyzes starch is
   a) pepsin. b) nitrogenase.
   c) lactase. d) amyloglucosidase.

3. Starch is converted to _____ and finally to glucose,
   a) raffinose b) fructose
   c) maltose d) sucrose

4. A _____ defatted sample is used for the estimation of starch.
   a) 0.7 mg b) 7.5 mg c) 57 mg d) 75 mg

5. The ingredients added to the sample before transferring to the autoclave were
   a) sulfuric acid and hydrochloric acid.
   b) water and sodium hydroxide.
   c) acetic acid and alcohol.
   d) alcohol and water.

6. The temperature maintained in the autoclave was
   a) 56°C. b) 126°C. c) 226°C. d) 26°C.

7. The pressure in kg cm^{-2} maintained in the autoclave for starch estimation was
   a) 2.18. b) 1.34. c) 4.34. d) 8.52.

8. The starch was _____ after the autoclave treatment.
   a) sterilized b) diluted
   c) distilled d) gelatinized

9. The enzyme added after autoclaving the flour sample was
   a) lactase. b) nitrogenase.
   c) amyloglucosidase. d) diastase.

10. The contents were incubated for _____ after adding the enzyme.
    a) 10 min b) 5 h c) 2 h d) 20 m

11. The contents were incubated at
    a) 5°C. b) 100°C. c) 55°C. d) 15°C.

12. The reagent _____ was added for incubation of starch.
    a) potassium permanganate b) phenolic resins
    c) phenol-sulfuric acid d) sodium acetate buffer

13. The percentage of starch with the following data is

Concentration of standard	25 µg mL^{-1}
Absorbance of standard	0.22
Sample mass	0.08 g
Absorbance of the sample	0.205
Volume taken for color development	0.1 mL
Factor for converting glucose to starch	0.9

    a) 72.7. b) 2.7. c) 27.0. d) 7.2.

MP 10. Soluble Sugars Estimation

1. To extract the soluble sugars ____% ethanol was used.
   a) 60 b) 70 c) 80 d) 90

2. Soluble sugars were estimated by using _____% phenol.
   a) 15 b) 25 c) 50 d) 5

3. A ____% sulfuric acid solution is used in the estimation of soluble sugars.
   a) 96 b) 98 c) 88 d) 86

4. A _____ sample of defatted flour was used for estimation of soluble sugars.
   a) 10 g b) 20 g c) 100 mg d) 5 mg

5. A quantity of ____ of ethanol was added to extract soluble sugars from the flour.
   a) 250 mL. b) 100 mL. c) 50 mL. d) 25 mL.

6. The absorbance of the _____ was read at 490 nm against a blank.
   a) golden-yellow. b) dark-green,
   c) scarlet-red. d) light-blue.

7. The percentage of soluble sugars in the sample with the following data is

Concentration of the standard	..	22 mg mL^{-1}
Absorbance of the standard	..	0.22
Sample mass	..	0.12 g
Absorbance of the sample	..	0.30
Extract taken	..	1 mL

a) 3.21. b) 1.23. c) 2.13. d) 21.3.

MP 11. Ash Estimation

1. A _____ sample was taken for the estimation of ash.
   a) 10 g b) 20 g c) 2 g d) 50 g

2. The sample was incinerated at
   a) 200°C. b) 600°C. c) 400°C. d) 100°C.

3. The sample was incinerated for _____ for estimation of ash.
   a) 30 min. b) 1 h. c) 4 h. d) 2 h.

4. The incinerated sample was cooled in a
   a) desiccator. b) thermostat,
   c) muffle furnace. d) vacuum pump.

5. The incineration of the sample was repeated until a _____ was obtained.
   a) constant volume b) constant mass
   c) good texture d) good color

6. The percentage of ash in the sample with the following data is

Mass of the crucible	..	6.0523 g
Mass of crucible + sample	..	8.9234 g
Mass of crucible + ash	..	6.0979 g

a) 1.95. b) 15.9. c) 5.9. d) 1.59.

MP 12. Tannin Estimation

1. Tannins are estimated as
   a) catechin equivalents. b) saponification value.
   c) diastatic activity. d) calorific value.

2. The reagent that is mixed with hydrochloric acid to make a reagent is
   a) methionine. b) butanol.
   c) methanol. d) ethanol.

3. The quantity of sample taken for tannin estimation is
   a) 500 mg. b) 100 mg. c) 50 mg. d) 10 mg.

4. The absorbance is read against the reagent blank at
   a) 5 nm. b) 50 nm. c) 100 ran. d) 500 nm.

Correct responses to the questions.

MP 1. Breaking Dormancy in Pearl Millet

1. d); 2. c); 3. b); 4. d); 5. c); 6. a); 7. d); 8. b); 9. a).

MP 2. Moisture Estimation

1. c); 2. a); 3. c); 4. a); 5. c); 6. d).

MP 3. Oil Estimation (Soxhlat)

1. c); 2. a); 3. d); 4. d); 5. b); 6. a); 7. d); 8. a).

MP 4. Protain Estimation

1. c); 2. d); 3. a); 4. c); 5. b); 6. d); 7. d); 8. a);
9. b); 10. c); 11. d); 12. b); 13. c); 14. d); 15. d);
16. d); 17. c); 18. c); 19. b); 20. d); 21. b); 22. c);
23. a); 24. b); 25. d); 26. c); 27. d); 28. a); 29. a);
30. c); 31. b).

MP 5. Lyaina Estimation

1. c); 2. b); 3. a); 4. d); 5. b).

MP 6. Amino Acids Estimation

1. c); 2. c); 3. c); 4. c); 5. c); 6. d).

MP 7. Mathionina and Cystine Estimations

1. c); 2. d); 3. b); 4. a); 5. d); 6. a); 7. c); 8. a);
9. d); 10. b).

MP 8. Tryptophan Estimation

1. b); 2. d); 3. d); 4. a); 5. b); 6. c); 7. d); 8. b); 9. a).

MP 9. Starch Estimation

1. c); 2. d); 3. c); 4. d); 5. d); 6. b); 7. b); 8. d);
9. c); 10. c); 11. c); 12. d); 13. a).

MP 10. Soluble Sugars Estimation

1. c); 2. d); 3. a); 4. c); 5. d); 6. a); 7. c).

MP 11. Ash Estimation

1. c); 2. b); 3. d); 4. a); 5. b); 6. d).

MP 12. Tannin Analysis

1. a); 2. c); a. a); 4. d).