APPENDICES

Appendix 1

Score card for the sensitivity threshold test

Name : Date :

You receive a series of bowls with solutions of sweet and bitter tastes. Start with bowl one and continue with 2, 3, 4 etc. Give intensity scores, i.e., rank the solutions according to the intensity of the taste.

Intensity score:

0 = None or the taste of pure water
? = Different from water, but taste quality not identifiable
X = Threshold very weak or taste identifiable
1 = Weak taste
2 = Mild taste
3 = Strong taste
4 = Very strong taste
5 = Extremely strong taste

For sweet solutions		For bitter solutions	
Bowl No.	Intensity scores	Bowl No.	Intensity scores
1		1	
2		2	
3		3	
4		4	
5		5	
6		6	
7			
8			
9			

Appendix 2

Score card for the triangle test

Name : Date :

Product :

Two of these 3 samples are identical, the third is different. Taste the samples and identify the odd sample.

Code	Check odd sample	Comments

Appendix 3

Score card for the composite scoring test

Name : Date :

Product :

Characteristics	Scores	Samples
Appearance	5	
Texture/mouthfeel	5	
Taste	15	
Leafy flavour	10	
After taste	10	
Acridity (irritation in the throat)	5	
Total :	50	

Comments:

Appendix 4

Card for the hedonic scale

Name : Date :

Product :

Taste these samples and check how much you like or dislike each one.

	Samples
Like extremely ..	..
Like very much ..	..
Like moderately ..	..
Like slightly ..	..
Neither like nor dislike ..	..
Dislike slightly ..	..
Dislike moderately ..	..
Dislike very much ..	..
Dislike extremely ..	..

Appendix 5

Reagents for the estimation of phosphorus

1. Molybdate solution: 25 g of ammonium molybdate was dissolved in 400 ml of water. Five hundred millilitres of 10 N sulphuric acid was added and the volume was made up to one litre with water.
2. Amino-naphthol sulphonic acid solution: 0.5 g of 1 amino -2 naphthol-4 sulphonic acid, 30 g of sodium bisulphite and

6 g of sodium sulphite were dissolved in water. The volume was made up to 250 ml. It was allowed to stand overnight and then filtered.

3. Standard phosphate solution: 0.4389 g of potassium diphydrogen phosphate was dissolved in water. Ten millilitres of 10 N sulphuric acid was added and the volume was made up to one litre with water. One millilitre of the solution contained 0.1 mg phosphorus.

Appendix 6

Reagents for the estimation of total iron

1. Mixture of concentrated nitric acid and sulphuric acid (5:1).
2. Saturated potassium persulfate solution: 7 to 8 g of potassium persulfate was shaken with 100 ml of water in a glass stoppered bottle, and was stored in the refrigerator.
3. 3 N potassium thiocyanate solution: 146 g of potassium thiocyanate was dissolved in water and volume was made up to 500 ml.
4. Standard iron solution: 0.702 g crystalline ferrous ammonium sulphate was dissolved in 100 ml of water, 5 ml of concentrated sulphuric acid was added to this and the solution was warmed slightly. Saturated potassium permanganate solution was then added, drop by drop until one drop produced a permanent pink colour. It was then transferred to one litre volumetric flask with rinsings and diluted to the mark. This solution contained 0.1 mg of ferric iron per millilitre.

Appendix 7

Reagents for the estimation of soluble and ionizable iron

1. Pepsin hydrochloric acid: 5 g of pepsin (1:10,000) was dissolved in 1000 ml of 0.1 N hydrochloric acid.
2. Hydroxylamine hydrochloride: 10 g of hydroxylamine hydrochloride was dissolved in 100 ml of double distilled water.
3. Acetate buffer: To 8.3 g of anhydrous sodium acetate was added 12 ml glacial acetic acid and the volume made up to 100 ml with double distilled water (pH 4.2).
4. Alpha alpha dipyridyl solution: 100 g of alpha alpha dipyridyl was dissolved in 100 ml of 3% acetic acid.
5. Acidified potassium permanganate: 3 g of potassium permanganate was dissolved in 100 ml of double distilled water. At the time of use, 8 ml of potassium permanganate solution was added to 5 ml of 0.5 N hydrochloric acid solution and the volume was made up to 20 ml.
6. Ascorbic acid: 20 g of ascorbic acid was dissolved in 100 ml double distilled water. This reagent was stored in the refrigerator.

7. Standard iron solution: 99.57 mg of ferrous sulphate with 7 molecules of water was dissolved in 100 ml of double distilled water. This solution contained 200 mcg of iron/ml. It was prepared fresh daily.
8. Working standard iron solution: One millilitre of the stock solution was diluted to 100 ml with double distilled water. This solution contained 2 mcg of iron/ml.
9. 6 N hydrochloric acid solution.
10. 0.1 N, 0.4 N and 0.5 N sodium hydroxide solutions.

Appendix 8

Preparation of the column for carotene estimation

Magnesium oxide was activated in an oven at 100°C for 3 to 4 h. It was then mixed with hyflo supercel in 1:1 ratio by weight. Glass wool was kept at the base of the column. Small amount of mixed adsorbant was added to the column and pushed down twice or thrice with a plunger. Then adsorbant was added again and the procedure was repeated till the length of the column was 10 cm. One centimetre layer of anhydrous sodium sulphate was placed over the adsorbant. The column was wetted with hexane and the top of the column was always kept covered with a layer of hexane/solvent during the entire procedure.

Appendix 9

Reagents for the estimation of thiamine

1. Sodium hydroxide, 15%: 15 g of sodium hydroxide was dissolved in water. The solution was cooled and diluted to 100 ml.
2. Potassium ferricyanine solution, 1%: One gram of potassium ferricyanide was dissolved in water and diluted to 100 ml. It was stored in a dark bottle in the refrigerator.
3. Sulphuric acid solution, 0.1 N: 2.8 ml concentrated sulphuric acid was diluted to one litre with water.
4. Sodium acetate solution, 2.5 M: 205 g of anhydrous sodium acetate was dissolved in water and diluted to one litre.
5. Enzyme preparation: 5 ml of enzyme suspension was prepared by the addition of 150 mg of taka diastase and 75 mg of papain to 5 ml of acetate buffer.
6. Potassium chloride solution, 25%: 250 g of potassium chloride was dissolved in water and diluted to one litre.
7. Base exchange silicate: For preparation of activated decalso, about 100 g of decalso was kept in 3% solution of hot acetic acid for 15 min. The acid was drained off and decalso was washed with 250 ml of hot 25% potassium chloride solution, letting this remain for 10 to 15 min before draining off. It was repeated once again. Then decalso was washed with

several portions of hot water until the hot water extract was free of chloride as shown by silver nitrate test. Decalso was dried at room temperature and then in the oven below 100°C and stored in a stoppered bottle.

8. Stock thiamine solution: 100 mg of thiamine hydrochloride was dissolved in 25% ethanol and the volume was made up to one litre with 25% ethanol.

9. Working standard thiamine solution: 5 ml of the stock thiamine solution (warmed to room temperature) was diluted to 100 ml with water. Four millilitres of this intermediate concentration was diluted to 100 ml with 0.1 N sulphuric acid. It was prepared fresh.

10. Quinine sulphate solution: 100 mg of quinine sulphate was dissolved in 0.1 N sulphuric acid and diluted to one litre with 0.1 N sulphuric acid. For working quinine sulphate solution, 3 ml of this solution was diluted to one litre with 0.1 N sulphuric acid. The solution was stored in a brown bottle.

Appendix 10

Reagents for the estimation of riboflavin

1. Sulphuric acid, 0.1 N.

2. Sodium acetate solution, 2.5 M: 205 g of anhydrous sodium acetate was dissolved in water and the volume was made up to one litre.

3. Potassium permanganate, 4%: 4 g of potassium permanganate was dissolved in 100 ml of water. It was prepared fresh daily.

4. Hydrogen peroxide, 3%: 30% hydrogen peroxide was diluted to 1:10 with water.

5. Riboflavin standard: 50 mg of riboflavin was dissolved in 1500 ml of water to which 2.4 ml glacial acetic acid was added in a 2 litre flask. It was warmed to aid solution. The solution was cooled and volume was made up to 2 litres. It was stored under toluene in amber bottle in refrigerator.

   For the preparation of working standard solution 20 ml of the stock riboflavin solution was diluted to 50 ml with water. Ten millilitres of this solution was diluted to 400 ml with water. Working standard was prepared fresh daily (one millilitre = one microgram riboflavin).

6. Sodium fluorescein solution: Stock solution contained 12.5 mg of sodium fluorescein in 250 ml of water. Working solution was prepared by diluting one millilitre stock solution to 500 ml with water.

Appendix 11

Reagents for the estimation of available lysine

1. Buffer, pH 8.5: 8% (W/V) of sodium bicarbonate and 8% (W/V) of disodium carbonate solutions were mixed in the ratio of 19:1 (V/V).
2. Phenolphthalein indicator, 1%: One gram of phenolphthalein was dissolved in 100 ml of 50% ethanol.
3. Stock DNP-lysine hydrochloride solution: 100 mg of DNP-lysine HCl was dissolved in 100 ml of N hydrochloric acid.
4. Working DNP-lysine hydrochloride solution: 3 ml of the stock solution was diluted to 100 ml in N hydrochloride acid. The working solution was prepared daily.

Appendix 12

Reagents for the estimation of hepatic protein

1. Phosphate buffer (pH 7.0): M/15 disodium phosphate was prepared by dissolving 9.4667 g of disodium phosphate in 1000 ml of distilled water. M/15 potassium acid phosphate was prepared by dissolving 9.0727 g of potassium acid phosphate in 1000 ml of distilled water. 611 ml of M/15 disodium phosphate was mixed with 389 ml of M/15 potassium acid phosphate to prepare 1000 ml of phosphate buffer.
2. Reagent A: 2.0 g of sodium tartrate was dissolved in 100 ml of 0.1 N sodium hydroxide.
3. Reagent B: One gram of copper sulphate was dissolved in 2.0% sodium tartrate solution. The solution was prepared fresh.
4. Reagent C, Alkaline copper reagent: 50 ml of Reagent A was mixed with one millilitre of Reagent B. The solution was prepared fresh.
5. Folin ciocalteau reagent: Commercial folin ciocalteau reagent was diluted 2 fold to make it to 1 N.
6. Standard bovine serum albumin (BSA): 100 mg of BSA was dissolved in 100 ml of 0.1 N sodium hydroxide.
7. Working BSA standard: One millilitre of stock standard was diluted to 10 ml with 0.1 N sodium hydroxide.

Appendix 13

Reagents for the estimation of serum total protein

1. Sulphate-sulphite solution: 208 g anhydrous sodium sulphate and 70 g anhydrous sodium sulphite were dissolved in 900 ml of acidified water (2 ml concentrated sulphuric acid to 900 ml distilled water) and volume was made up to one litre with water.

2. Stock biuret reagent: 45 g sodium potassium tartrate was dissolved in 0.2 N sodium hydroxide. 15 g copper sulphate, $CuSO_4.5H_2O$, was added and stirred until solution was complete. Five grams of potassium iodide was added to it and volume was made up to one litre with 0.2 N NaOH.
3. Biuret reagent for use: 200 ml of stock reagent was diluted to one litre with 0.2 N sodium hydroxide containing 5 g potassium iodide/l.
4. Tartrate iodide solution: 9 g of sodium potassium tartrate was dissolved in 0.2 N sodium hydroxide containing 5 g potassium iodide/l.
5. Bovine serum albumin standard: 400 mg of bovine serum albumin was dissolved in 100 ml of 0.2 N sodium hydroxide.

Appendix 14

Reagents for estimation of serum urea

1. Trichloroacetic acid, 100 g/l.
2. Stock diacetylmonoxime, 25 g/l in water.
3. Stock thiosemicarbazide, 2.5 g/l in water.
4. Acid ferric chloride solution: One millilitre of sulphuric acid was added to 100 ml of a ferric chloride solution containing 50 g/l in water.
5. Acid reagent: 10 ml of orthophosphoric acid was added to 80 ml sulphuric acid and 10 ml acid ferric chloride solution which were added to one litre of water and mixed.
6. Colour reagent: 300 ml of acid reagent was added to 200 ml of water, 10 ml of stock diacetylmonoxime and 2.5 ml of stock thiosemicarbazide.
7. Urea standards: A stock urea standard solution was prepared by dissolving 3.0 g of urea in 100 ml of distilled water. Working standard solutions were prepared by diluting one to 10 ml of the stock standard solution to 100 ml providing standards of 30 to 300 mg/100 ml concentrations.