Material and Methods
MATERIALS AND METHODS

For the sake of convenience this chapter is described under the following heads:

I. PHYSICO-CHEMICAL COMPOSITION OF FRUITS OF IMPORTANT BANANA CULTIVARS
   A. Comparative study of thirteen cultivars
   B. Determination of the physico-chemical composition of Champa and Kabuli in different months of the year

II. STUDIES ON DEVELOPMENTAL PHYSIOLOGY
   A. Comparative developmental physiology of Champa and Kabuli
   B. Comparative developmental physiology of the cultivar, Martaman in two different seasons (summer and winter)

III. POST-HARVEST PHYSIOLOGY
   A. Physico-chemical changes during ripening in Champa, Kabuli and Martaman
   B. Post-ripening physico-chemical changes in seven cultivars

IV. STATISTICAL ANALYSIS
I. PHYSICO-CHEMICAL COMPOSITION OF FRUITS OF IMPORTANT BANANA CULTIVARS

A. Comparative study of thirteen cultivars

The following cultivars were selected for the present study. 1) Champa, 2) Kabuli, 3) Giant Governor, 4) Lacatan, 5) Martaman, 6) Martaman (Madras), 7) Jurmony Kanthali, 8) Deshi Kanthali, 9) Kalibow, 10) Amritsagar, 11) Agniswar, 12) Bichakala (Dimley kala), 13) Anupam.

A short introductory note on the above mentioned cultivars is given below:

1) Champa (Musa AAB group): Syn. Poovan (Tamil Nadu), Lavelche (Maharastra), Raspura Chackrakel (Andhra), Palyanandan (Kerala), Kadali (Madurai), Fill Basket or Mysore (Trinidad). This is the foremost commercial cultivar of Tamil Nadu, Andhra Pradesh and West Bengal. The fruit is small, skin is yellow and thin, flesh firm, sweet but has a subacid taste, its keeping quality is high.

2) Dwarf Cavendish (Musa AAA group): Syn. Kabuli (West Bengal), Lotan (Maharastra), Vamankel (Andhra Pradesh), Pachavazhi or Mauritious, Kuzhi Vazhai (Tamil Nadu). It is grown in small pockets of 24 Parganas. The plant is very dwarf, fruits large, curved, skin thick and greenish but becomes yellow in winter. Flesh soft and sweet with good flavour.
3) **Giant Governor (Musa AAA group):** It belongs to the Giant Cavendish group. The plant is medium dwarf, fruit large, skin thick, greenish to dull yellow in colour, flesh firm and sweet with good flavour and keeping quality.

4) **Lacatan (Musa AAA group):** It belongs to the Giant Cavendish group. The plant is medium-dwarf, fruit large, skin thick, greenish in colour, flesh firm and sweet.

5) **Martaman (Musa AAB group):** Syn. Rastheli (Tamil Nadu), Mukheli (Maharastra), Mulbhog (Bihar), Amrapani (Andhra), Poovan (Kerala) and Silkfig (Trinidad). It is the choicest cultivar in West Bengal. It is grown in the districts of Midnapore, 24-Parganas, Nadia, Coochbehar and Jalpaiguri. The plant is tall, fruits are medium size, skin very thin, ivory yellow in colour, flesh firm, sweet with excellent flavour.

6) **Jurmony Kanthali (Musa xxx group):** It has been introduced from Assam. The plant is very tall, hardy and robust in stature. The fruit is medium, skin thick, greenish to dull yellow, flesh sweet and firm.

7) **Deshi Kanthali (Musa xxx group):** The plant is very tall, hardy and robust. The fruit is small, skin medium thick, light yellow colour, flesh firm and sweet.

8) **Kalibow (Musa xxx group):** The plant is very tall, hardy and robust. The fruit is small, skin thin dull yellow colour, flesh firm and very sweet.

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xxx = Ploidy level not known
9) **Amritsagar** (*Musa AAB group*): This is considered one of the choicest cultivars of India. The plant is medium tall, fruit large, skin medium thick, golden yellow in colour. Flesh is firm, sweet with excellent flavour.

10) **Agniswar** (*Musa AAA group*): The plant is tall, fruit small, skin medium thick, bright red in colour, flesh firm and medium sweet, flavour not so good.

11) **Bichakala** (*Dimley kala*): The plant is tall. The fruit is large, skin thick, yellow in colour, flesh soft and sticky, pulp contains a good number of seeds, pulp is very sweet with medium flavour.

12) **Anupam** (*Musa AAA group*): Robust, tall, moderately stooling, with dark reddish violet pseudostem. Fruit large, curved, slightly angular, pulp pale yellow, firm, fairly sweet, good flavour.

**Physical characters**

Fully mature fruits of all the cultivars except *Martaman* and *Dimley* were collected from the Banana Research Station, Chinsurah and the rest were purchased from local cultivators in the middle of February, 1973. All the fruits were stored at room temperature (25 ± 2°C) and after they reached the right edible stage the different physical characters were studied.
i) Length: The length of fruit was measured with a centimeter scale.

ii) Diameter: Diameter of the fruit was thus measured with slide callipers.

iii) Weight: Fresh weight of the whole fruit and peel were recorded in grams.

Biochemical characters

Dry matter: A known amount of composite pulp was kept in an oven at 70°C till a constant weight was reached. The percentage of dry matter was calculated on fresh weight basis.

Total soluble solids: Ten grams of fresh sample were macerated and mixed with 100 ml of distilled water and T.S.S. were measured with a hand refractometer.

Titratable acidity: Ten grams of sample were macerated and mixed with 100 ml of distilled water and after filtration it was titrated against 0.1 N NaOH using phenolphthalein as an indicator. The results were expressed in ml of 1N NaOH required per 100 g fresh pulp.

Ascorbic acid (Vitamin C): One gram of fresh pulp was taken in a beaker in which 25 ml of metaphosphoric acid solution were added. It was macerated and 10 ml of distilled water were added and stirred for 5 minutes and then titrated rapidly with the indophenol solution from the semi-microburette. The results were expressed in terms of milligrams of ascorbic acid per 100 g fresh pulp. (A.O.A.C, 1970).
Estimation of reducing sugars: Twenty five grams of well macerated pulp were taken in a 250 ml conical flask and 100 ml of distilled water were added and the contents were stirred for 45 to 50 minutes. The solution was then clarified with slight excess of neutral lead acetate solution and filtered through a Whatman No.1 filter paper. The excess of lead acetate was removed from the solution by adding potassium oxalate solution and then filtered through a Whatman No.42 filter paper. The final volume was made upto 250 ml in a volumetric flask.

Five ml of Fehling's solution No. 1 and five ml of Fehling's solution No. 2 were taken in a 300 ml conical flask and 30 ml of distilled water were added to it. The clarified solution was taken in a 50 ml burette and the titration was continued until the red precipitate of cuprous oxide appeared and the supernatant liquid was colourless. The titration was done in a boiling condition using methyl blue as indicator.

Estimation of total sugars: For this 50 ml of the clarified lead acetate free solution was taken in a 300 ml conical flask to which 20 ml of distilled water and 10 ml of concentrated HCl were added. The contents were heated in a water bath for about 30 minutes at 60-65°C. Then the solution was cooled and neutralized with sodium hydroxide using phenolphthalein as indicator, and the volume was made upto 100 ml. The reducing sugar of this solution was estimated as before. The difference between the percentage of total sugars (after hydrolysis) and
the percentage of reducing sugars gave the value for non-reducing sugars (Lane and Wynon, 1923).

Estimation of total nitrogen: Five hundred milligrams of dried sample were taken in a 300 ml digestion flask and 1 gram of anhydrous sodium sulphate and a pinch of copper sulphate were added to it. Then 15 ml of conc. H₂SO₄ were added and digested for one hour until the colour became greenish and transparent.

To the digested sample 100 ml of distilled water were added and a piece of blue litmus paper was kept in the digestion flask. Then 100 ml of 40 percent NaOH were poured slowly and heated. The ammonia evolved was absorbed in 0.1 N H₂SO₄ in which 2 drops of Weslow's indicator were added. The excess H₂SO₄ was titrated against a standard NaOH solution.

Calculation: 1 ml of 0.1 N H₂SO₄ = .0014 g of nitrogen.

For the determination of the percentage of protein the value of total nitrogen was multiplied by 6.25.

Estimation of total phosphorus: Five hundred milligrams of dried samples were taken in a 100 ml volumetric flask and 4 ml of tri-acid mixture (HNO₃ : H₂SO₄ : HClO₃, 10 : 2 : 1, V / V / V) were added to it and digested on a sand bath till the contents were colourless. Then the digested material was cooled and the volume was made upto 100 ml. From this extract, the total phosphorus was estimated by the Vanado-molybdate method as stated by Jackson (1973). Five millilitres of the phosphorus containing
stock solution were pipetted into a 25 ml volumetric flask, and the pH was adjusted or neutralised with 4N Na₂CO₃ and 4N HCl, 2, 4-paranitrophenol being used as an indicator. Then 10 ml of vanado-molybdate solution were added by means of a volumetric pipette and the volume was made to 25 ml with double distilled water. The temperature throughout the determination was maintained at 25 ± 5°C. The solution was mixed thoroughly and a yellow colour developed. The contents were kept overnight and the next day the colour intensity was read colorimetrically by using a blue filter (450m\(\mu\) - 500m\(\mu\)). The quantity of phosphorus was determined from the standard curve, prepared with known quantities of phosphorus.

**Estimation of total potassium**: The total potassium was determined using a Beckman Flame Emission Photometer following the method of Jackson (1973). Hundred milligrams of finely powdered sample that had been dried at 100°C for 2 hours were placed in a 30 ml platinum crucible. The crucible, with lid covering nine-tenth of the top, was placed on a sand bath at a temperature of 200-225°C, and the acids were evaporated to dryness. The solution was not heated vigorously to avoid spattering. After decomposition the ions which interfered were removed by the NH₄OH separation. The resulting solution was treated with conc. HNO₃ to remove the ammonium salt. The residue was taken in 25 ml of 0.4N HCl for the determination of flame emission. For this the instrument was allowed to warm up for a period of 10-15 minutes prior to putting it in operation. The determination
of K required the red sensitive photoelectric cell whose major flame emission line occurred between 600-1000 m\(\mu\). During the warming up period, the various standard solutions and sample solutions were poured into 5 ml beakers and a suitable record sheet was drawn up. A calibration curve was prepared for the element in 0.4N HCl base electrolyte solution. For this, a series of stock solutions containing 1000 ppm K (1.907 g of KCl was dissolved in 1 litre of water) were prepared. The blank containing all salts that would be present in the analytical determination, except the element to be determined, is called the base electrolyte solution. This must be exactly the same in the standard and the test samples, to provide the same interferences and viscosity effects. The concentration of the element was expressed as mg per 100 ml solution. Calculation of concentration was based on the following relationship:

\[
\% \text{ element} = \text{mg of element per 100 ml} \times \frac{0.120}{\text{weight of sample (g)}}
\]

Estimation of total calcium: Total calcium was determined by versene method after removal of ammonium salts as suggested by Jackson (1973).

Five hundred milligrams of dried samples were digested in a triacid mixture and the volume was made up to 250 ml in a volumetric flask. Then 50 ml of the solution were taken in a silica basin and neutralised with 0.1N NaOH. Five millilitres of KOH solution and 0.3 g of murexide indicator powder were added to the solution. Then the amount of calcium in the solution was
determined with 0.0200N versene solution to a purple end point. Murexide, also known as ammonium purpurate, formed an orange red complex with calcium ions at pH 12, which was converted to a red violet colour when the calcium ions were completely complexed by versene. The colour change was gradual, making it imperative to compare the end point colour with a standard. The end point could be reached from either direction, and back titration with standard calcium solution was possible.

Calculation of calcium concentration was based on the relationship:

\[
\% Ca = \frac{ml \text{ versene} \times 0.0481}{\text{weight of sample} (g)}
\]

Estimation of total magnesium: Total magnesium and calcium contents could be determined by versene titration with Eriochrome black T indicator, the total calcium determined separately, was subtracted, thus giving the value for total magnesium.

Eriochrome black T dye forms a wine-red coloured complex with Mg, but changes to blue when all the Mg ions have been removed by the versene; the titration is much more satisfactory than that with murexide. The formation of the Mg complex is optimum at pH 10 in the presence of NH₄Cl and NH₄OH. The calcium is complexed by versene before the magnesium, and therefore the titration value gives total Mg plus Ca.

In the present study the murexide indicator remaining after the Ca titration with versene solution was destroyed by the addition of a few drops of bromine water and the solution
was then acidified to bring the Mg(OH)₂ into solution. A solution containing 1 g of NH₄Cl was added and then sufficient NH₄OH was added to bring the final solution to pH 10. Next, 5 drops of Erichrome black T indicator solution and 1 ml of 2% NaCN solution were added. The solution was then titrated with 0.0100 N versene solution to a blue end point. The titration was a measure of the total Mg plus Ca in the solution. To obtain the percentage of Mg in the sample, the Ca equivalent was subtracted.

Calculation:

\[
\text{meq Mg + Ca per g sample} = \frac{\text{ml versene}}{\text{(Erichrome)}} \times \frac{0.024}{\text{wt. sample g}}
\]

\[
\text{meq Ca per g sample} = \frac{\text{ml versene}}{\text{(Murexide)}} \times \frac{0.024}{\text{wt. sample g}}
\]

Then, \( \% \text{ Mg} = \frac{\text{meq Mg + Ca}}{\text{(per g sample)}} - \frac{\text{meq Ca}}{\text{(per g sample)}} \times 1.216 \)

B. Determination of the physico-chemical composition of Champa and Kabuli in different months of the year

Two cultivars Champa and Kabuli were selected for this experiment. The samples were collected at an interval of one month from the Banana Research Station, Chinsurah. The experiment was conducted for two years. It was started from the month of August, 1972 and was continued up to July, 1974.

The samples were collected and kept at room temperature until they attained the most appropriate edible stages.
Physical characters

All the physical characters viz. Length, diameter of fruits, weight of peel and pulp were recorded according to the method mentioned in the previous chapter.

Biochemical characters

For the determination of the chemical composition; the percentage of dry matter, total soluble solids, titratable acidity, ascorbic acid, reducing and non-reducing sugars, protein, phosphorus, potassium, calcium and magnesium the methods followed were the same as those described earlier.

II. STUDIES ON DEVELOPMENTAL PHYSIOLOGY

A. Comparative developmental physiology of Champa and Kabuli

The study on the developmental physiology of Champa was done on 20.3.74. The flowers were tagged on 20.3.74.

The date of emergence of the 1st hand was 20.3.74.

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20.3.74
24.3.74
26.3.74
28.3.74
29.3.74
30.3.74
31.3.74
4.4.74
Each hand contained 20 fruits. The bunches were harvested after 105 days and were kept at room temperature. The sampling was done at intervals of 7 days.

The study on Kabuli was done from 14.5.74, the flowers were tagged on the first day of emergence.

The date of emergence of the 1st hand was 14.5.74.

- 2nd: 16.5.74.
- 3rd: 17.5.74.
- 4th: 17.5.74.
- 5th: 18.5.74.
- 6th: 18.5.74.
- 7th: 18.5.74.

The number of fruits in the hands was not uniform. The fruits matured after 105 days. The bunches were harvested and kept at room temperature and sampled at an interval of 7 days.

**Physical characters**

Weight of the fruit, pulp and peel were recorded following the methods mentioned in the previous chapter.

**Biochemical characters**

A known amount of composite pulp portion was used for the estimation of dry matter content. A weighed amount of the pulp was blended with distilled water and then centrifuged
for ten minutes. The supernatant was made up to a known volume and titratable acidity, ascorbic acid and T.S.S. contents were determined from the diluted supernatant following the methods described earlier.

Estimation of reducing, non-reducing and total sugars and reserve polysaccharides:

The flesh of the fruits was chopped into small bits and 5 g fresh weight of these were placed in a conical flask containing 25-30 ml of 80% ethanol and was heated on a boiling water bath for a few minutes to inactivate the enzymes. The contents of the conical flask were refluxed for 6 hours using an air condensor. After refluxing the contents were filtered and washed several times with 80% ethanol and the filtrate was made up to the desired volume. An aliquot (containing 1 g equivalent fresh weight of pulp) was taken in a 100 ml beaker and placed on a boiling water bath to evaporate the alcohol completely. The contents of the beaker were not allowed to dry up completely. After the alcohol was removed, 1 ml of saturated basic lead acetate solution was added to it to precipitate the colloidal substances and colouring matters. It was filtered through Whatman No. 1 filter paper in a conical flask containing 3 ml of saturated di-sodium hydrogen phosphate solution which precipitated the excess lead. This was filtered again through Whatman No. 42 filter paper in a 100 ml volumetric flask and the volume was
made up to the mark with distilled water. Requisite aliquots from clarified extracts were taken for the estimation of reducing sugars following the modified copper reduction method described by Somogyi (1945, 1952).

Estimation of soluble nitrogen: The samples of soluble nitrogen preserved in the refrigerator were taken in a clean mortar and pestle and made into a paste with addition of a little amount of distilled water. The macerated material was transferred into a Buchner funnel for filtration through Whatman No. 1 filter paper. The extraction was done under reduced pressure applying suction by the help of a filter pump. The contents were thoroughly washed and 19 ml of the extract were collected into a test tube fitted to the Buchner funnel. Frothing was prevented by addition of one or two drops of capryl alcohol to the material. The filtrate was taken in a 25 ml volumetric flask and made up to the volume by addition of distilled water. Then an aliquot was taken therefrom in a beaker followed by an addition of 1 ml trichloroacetic acid for the precipitation of the proteins in the extract. The solution was stirred frequently and then kept for half an hour. Thereafter it was filtered through Whatman No. 42 filter paper and 5 ml of the filtrate were taken into a microkjeldahl digestion flask for digestion. After digestion the content of the digestion flask was transferred to the microdistillation flask and 10 ml of 40% NaOH and 6.6% sodium thiosulphate were added. It was then distilled by steam and
the ammonia evolved was collected in a 100 ml conical flask containing 10 ml of 0.02 N H₂SO₄ and 2 drops of Weslow's indicator. Steam distillation was carried out for 13 to 15 minutes for complete evolution of ammonia. The excess of 0.02 N H₂SO₄ was titrated against 0.02 N NaOH solution. The difference gave the quantity of 0.02 N H₂SO₄ that reacted with the evolved ammonia.

\[ 1 \text{ ml of } 0.02 \text{ N } H₂SO₄ = 0.0028 \text{ g of nitrogen} \]

**Estimation of total nitrogen**: The sample which was kept in the oven for drying was weighed. The dry material was powdered. Thirty mg of the composite sample were then weighed accurately and transferred to the microdigestion flask followed by 5 ml of distilled water, a pinch of sodium sulphate and copper sulphate (3:1) and 3 ml of conc. H₂SO₄. Then it was digested till the contents became colourless. The rest of the method was similar to that of estimation of soluble nitrogen.

Both soluble and total nitrogen were expressed on dry weight basis. The protein nitrogen was obtained by subtraction of the value of soluble nitrogen from total nitrogen.

**Estimation of minerals**: The concentrations of total potassium, calcium and magnesium were determined by the methods discussed in the previous chapter.
B. Comparative developmental physiology of the cultivar, Martaman in two different seasons (summer and winter)

This study was done in two different seasons viz. summer and winter. In the first season the date of emergence of hands were as follows:

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<th>1st hand</th>
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There were 14 fruits in each hand and sampling was done at an interval of 7 days. The bunch was harvested after 118 days of emergence i.e. on 23rd May, 1974. After harvest the sampling was done at an interval of one day until the fruits were well ripened (edible stage).

Studies on the developmental physiology of the cultivar Martaman which matured in the winter season were conducted during the period late August to December.

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There were 18 fruits in each hand and sampling was done at an interval of 7 days. The bunches were harvested after 112 days of emergence i.e. on 11th December, 1974, and the matured samples were kept for ripening at prevailing room temperature (21°C ± 2). The sampling during the post-harvest period was done at an interval of one day until the fruits were fully ripened (edible stage).

All the physical and chemical characters of the fruit were analysed following the methods mentioned in the previous chapters.

III. POST-HARVEST PHYSIOLOGY

A. Physio-chemical changes during ripening in Champa and Kabuli

A comparative study of the physico-chemical changes in fruits of three cultivars Champa, Kabuli and Martaman was taken up during the period March, April and May, 1974, and after harvesting the samples were kept at room temperature and sampling was done at an interval of one day, till they reached the edible stage.
Physical characters

Length, diameter of fruits, weight of peel and pulp of the fruits were recorded according to the methods mentioned in the previous chapters.

Biochemical characters

The methodology for the determination of percentage of dry matter, total soluble solids, titratable acidity, ascorbic acid, reducing and non-reducing sugars, protein, phosphorus, potassium, calcium and magnesium was the same as described earlier.

B. Post-ripening physico-chemical changes in seven cultivars

For post-ripening (from edible stage onwards) studies of fruits of seven cultivars, were conducted with fruits collected from the Banana Research Station, Chinsurah, and from the local cultivators. After ripening, when the fruits had reached the edible condition, they were kept at room temperature (28°C ± 2) for eight days. The experiment was started on 28th February, 1973 and sampling were done at an interval of 2 days. The study of the physical characters and biochemical analyses of the fruits were done following the methods described earlier.
IV. STATISTICAL ANALYSIS

Standard error: Each experiment was repeated two to three times and in each set there were 5-20 replications. The standard error of means was calculated by the following formula:

\[ S.E.(\bar{X}) = \sqrt{\frac{x^2 - n(\bar{x})^2}{n(n-1)}} \]

where \( x \) = individual variate, \( \bar{x} \) = mean and \( n \) = total number of replications.

Test of significance: For testing the significance of treatments, the analysis of variance technique (Fisher, 1948) was followed. L.S.D. (least significance difference) between the treatments has been calculated by the following formula

\[ \text{L.S.D.} = \sqrt{\frac{2 \times \text{error m.s.}}{r}} \times \text{table value 't' with error d.f. at 0.05 P and 0.01 P} \]

where \( r \) = number of replication

In case of interaction effects the analysis of variance was calculated from two-way table by the methods of Panse and Sukhatme (1967) and Cochran and Cox (1967).