Bulbils and tubers form the economically useful portions of yams. Single and double harvesting methods are practiced in yam collection. In single harvesting, the crop is harvested towards the end of season. Harvesting is done at any time after large scale, leaf-yellowing, sets in. In our state, as well as in other tropical regions, harvesting is done in January – February. Harvesting can be done any time from one month before senescence till 1-2 months after it. Delay in harvesting results in the deterioration of the tubers. Soil around the tuber is loosened and the tuber is then lifted without any bruises as this will lead to microbial infection during storage.

In double harvesting, tuber is detached from the plant after 4-5 months growth, without disturbing the roots. The second harvesting is done at the end of the season. Tubers produced in the second phase are amorphous with lots of buds on it. This practice is followed to produce large numbers of – setts - planting materials for the next season. The collected tubers are then washed carefully and shade dried. Well dried tubers are chipped and the flakes are then air dried in shade till maximum dehydration is effected. These flakes are then stored in airtight containers.

**Estimation of diosgenin**

The quantity of Diosgenin in samples was determined as described by Baccou et al., (1977) and Uematsu et al., (2000).

The dried flakes of tuber were oven dried at 70°C for 72 hours. 1 gram of the pulverized material was placed in a plastic tube (50 ml capacity, centrifugal grade) and 30 ml methanol was added to the tubes and left on the shaker over night. The extract was centrifuged at 3500 rpm for 18 minutes and the supernatant collected. Two subsequent extractions were carried out and the supernatants collected. The final volume was adjusted to 100 ml.

For analysis, two colour developing reagent solutions were prepared. Solution A consists of 0.5 ml p-anisaldehyde in 99.5 ml ethyl acetate. Solution B is made by mixing 50 ml
concentrated sulphuric acid with 50 ml ethyl acetate. 100µl of the methanol extract is taken in a tube and the methanol was evaporated under reduced pressure. The residue left behind was dissolved in 2ml of ethyl acetate and 1 ml each of reagents A and reagent B and stirred well. The test tube was placed in a water bath maintained at 60° C for 10 minutes to develop colour. It was then allowed to cool for 10 minutes in 25° C water bath. The absorbance of the coloured solution was measured in a spectrophotometer at 430 nm. As a reagent blank, 2ml ethyl acetate was placed in tube and assayed in similar manner.

For calibration curve, 2 – 70 µg standard diosgenin in 2 ml. ethyl acetate was used. Each sample was repeated thrice and the average was taken

**Protein estimation through Lowry’s method.**

This is a commonly used method to estimate protein in samples. It is very sensitive and quantities as low as 20µg of proteins can be measured. 100 milligrams of the dried and powdered tuber was macerated in pestle and mortar with 5 ml. of phosphate buffer (0.01 M, pH 7.6). This was then centrifuged at 8000 rpm for 20 minutes and the supernatant collected. The extraction process was repeated 4 to 5 times and the supernatants collected. The volume was made up to 50 ml using the buffer. To 1 ml of the extract, 1ml of 20% TCA was added and kept for an hour. It was then centrifuged at 8000 rpm for 20 minutes and the supernatant discarded. The pellet was then washed with acetone and centrifuged. The pellet obtained after discarding acetone supernatant was then dissolved in 5 - 20ml of 0.1N NaOH and mixed well to dissolve.

1ml of the above solution was then mixed with 5ml of freshly prepared alkaline copper sulphate reagent and mixed thoroughly. After 10 minutes, 0.5ml of Folin's reagent was added and allowed to develop colour for 30 minutes. The readings were taken in a colorimeter at 660nm. Each sample was screened thrice and the average value was taken. Reference curve was prepared using BSA (bovine serum albumin) in concentrations of 40 - 200µg/ml.
**Determination of starch**

Sugars are first extracted by treating the finely powdered tuber repeatedly with hot 80% (v/v) ethyl alcohol. The residue is then treated with cold perchloric acid to solubilise starch. After filtration, starch in the perchloric acid extract is hydrolysed to glucose in hot acidic medium which undergoes dehydration to hydroxymethyl furfural. This derivative then condenses with anthrone to give a blue coloured complex and is determined quantitatively by the intensity of colour developed.

0.2 gm of the dried sample was taken in a 50 ml centrifuge tube and added 20 ml of hot 80% alcohol. The tube was shaken for 10 minutes and centrifuged at 3000 rpm for 10 minutes. The supernatant was then decanted. The process was repeated 5-6 times to remove sugar contents. The residue left behind was then cooled by placing in ice water. To this, 6.5 ml of 52% perchloric acid was added with constant stirring using a glass rod. It was then centrifuged and the supernatant collected. The step was repeated 4-5 times and the supernatants were then pooled and the volume made up to 100 ml with water. This extract was then diluted further and 5 ml aliquots were taken in tubes and placed in cold water bath. To this, 10 ml of freshly prepared anthrone reagent was added, mixed and placed in boiling water bath for 7.5 minutes. After cooling the tubes in running tap water, the absorbance of the mixture was noted at 630 nm in a spectrophotometer. Standard curve was prepared using 0 – 100 µg glucose as described above.

**Estimation of calcium by Calcium – O – Chrysolphthaleine method.**

The sample preparation was done by wet digestion method. 1 gram of the oven dried and powdered material was taken in a 100 ml Kjeldahl flask. 25 ml of a mixture of concentrated HNO₃, conc. HClO₄ and conc. H₂SO₄ (3:2:1) were added and shaken well. The mixture was allowed to stand for 3-4 hours and later filtered through Whatman,s filter paper No. 40 into 100 ml volumetric flask. The residue on filter paper is washed with dilute HCl (1: 19). The volume is made to 100 ml with dilute HCl. The filtrate is stored in acid washed polythene bottles.

The calcium – o - cresolphthalein complexone (OCPC) method was originally reported by Schwartzenbach, et al., (1954). Stern and Lewis, (1957) later adapted this reaction to
calorimetric assay. Connerty and Briggs, (1966) demonstrated the use of 8-quinolinol to reduce magnesium interference. Calcium reacts with OCPC to form a purple complex. The sample was mixed with liquid A and liquid B supplied by the company (Flex reagent cartridge) for developing colour. Liquid A is glycine buffer at concentration of 0.22 mmol/L and liquid B consists of OCPC at 0.39 mmol/L and 8-Quinolinol at 6.60 mmol/L. The sample was mixed with the reagents A and B in 5µl:145 µl:33 µl proportion. The intensity of the colour is proportional to the concentration of calcium in the sample. The readings were taken with the help of Dimension clinical chemistry system which is automated for the calibrations and calculations. The system uses monochromatic light at 577 nm and 540 nm. Reference curves were prepared using 2-12 mg/dl CHEM 1 calibrator (cat.no. DC18A) supplied by the company, Dade Behring Inc. U.S.A. Each sample was repeated thrice and the average taken.

**Determination of phosphorous**

The sample preparation was done by wet digestion method. 1 gram of the oven dried and powdered material was taken in a 100 ml Kjeldahl flask. 25 ml of a mixture of concentrated HNO₃, conc. HClO₄ and conc. H₂SO₄ (3:2:1) were added and shaken well. The mixture was allowed to stand for 3-4 hours and later filtered through Whatman,s filter paper No. 40 into 100 ml volumetric flask. The residue on filter paper is washed with dilute HCl (1:19). The volume is made to 100 ml with dilute HCl. The filterate is stored in acid washed polythene bottles.

2 ml. of the digested sample extract was transferred to 25 ml volumetric flask. A few drops of 2, 4 – dinitrophenol indicator was added to this and the contents were neutralized with 4 N ammonia. Excess ammonia when present was neutralized with 2N H₂SO₄ and the volume made to two third of the flask with water. One milliliter of sulphomolybdate solution was then added followed by 0.5 ml of freshly prepared stannous chloride solution. The contents were then thoroughly mixed and the volume was made to 25 ml. with water. Absorbance of the blue coloured solution was recorded at 660 nm, within 4 to 20 minutes of preparation. Standard curve of phosphorous, containing 0.5 ppm to 1.5 ppm was prepared using the same procedure.