Chromatographic analysis of Simple Sugar in Banana and Chiku

Project Report

Under

DBT Star College Scheme
Department of Biotechnology, New Delhi

By

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Certificate

This is to certify that the work incorporated in the project report **Chromatographic analysis of Simple Sugar in Banana and Chiku** by Miss. Darandale Rutuja Nanasaheb, Miss. Darandale Swapnali Nanasaheb , Miss. Rajdeo Bhakti Sarjerao, Miss. Khilari Vaishnavi Subhash, Miss. Shinde Suvarna Annasaheb, are students of Arts, Commerce and Science College Sonai, Tal. Newasa, Dist. Ahmednagar. Affiliated to the Savitribai Phule Pune University Pune successfully completed project.

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Declaration

We hereby declare that the work done in this thesis entitled on **Chromatographic analysis of Simple Sugar in Banana and Chiku** is submitted to Department of Botany, Arts, Commerce and Science College Sonai. This project is completed under the DBT Star College Scheme and the supervision **Dr. B. J. Apparao** The works is original and not submitted in part or full by me or any other to this or any other University.

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INTRODUCTION

Carbohydrates are among the most abundant compounds in the plant world, and the analysis of sugars and sugar mixtures is of considerable importance to the food and beverage industries. A variety of chromatographic systems including paper and thin-layer chromatography, gas–liquid chromatography with flame ionization or mass spectrometric detection, and high-performance liquid chromatography (HPLC) can be used to separate and analyze them. Thin-layer chromatography (TLC) is a useful tool for the rapid separation of a wide range of compounds of biological interest. The classes of compounds which can be separated by TLC depend both on the sorbent material used for the layer and the selected solvent system. The majority of the workers have used silica gel for the sorbent layer with a fair degree of success. However, TLC has not been as successful for the separation of sugars as it has been for the separation of many other compounds. Paper chromatography has been the method of choice for many years for the separation of sugars. The major disadvantage of paper chromatography is that the time necessary to separate a series of sugars ranges from 24 hours to three or four days. When many samples must be analyzed this may become a serious problem.

Musa sapientum is commonly called banana is an herbaceous plant of the family *Musaceae*. It is known to have originated from the tropical region of Southern Asia. It is now cultivated throughout the tropics (Leslie and Cobley, 1976). Plant is cultivated primarily for its fruits and to a lesser extent for the production of fiber (Akinyosoye, 1991). It is also used as an ornamental plant. The *Musa sapientum* grows up to a height of about 2-8m with leaves of about 3.5m in length. The stem which is also called pseudo stem produces a single bunch of bananas before dying and replaced by new pseudo stem. The fruit grows in hanging cluster, with about twenty fruits to a tier and 3 – 20 tiers to a bunch. The fruit is protected by its peel which is discarded as waste after the inner fleshy portion is eaten. *Musa sapientum* fruits have been reported to prevent anemia by stimulating the production of hemoglobin in the blood. Its role to regulate blood pressure has been associated with the high content of potassium (Akinyosoye, 1991). Banana helps in solving the problem of constipation without necessary resorting to laxatives. Banana can cure heart burns stress, strokes, ulcers and many other ailments [Wath, and Brayer-Brand Wijk, 1962). The peels have been reported to be useful in making banana charcoal, an alternative source of cooking fuel in Kampala.

The peels in conjunction with other substances create a liniment for reducing the acuteness of the arthritis aches and pains (Kudan, 1962). Considering the upsurge in the prizes of commercial sugars and their increasing demand, this study was conducted to provide information about the *Musa sapientum* peel which is often ignore and considered as waste could be domesticated for proper utilization in an economical manner (Chandraju *et.al*., 2011).

Manilkara zapota, commonly known as sapodilla, sapota, chikoo, chico, naseberry is a long lived, evergreen tree native to southern Mexico, Central America and the Caribbean. It was introduced to the Philippines during Spanish colonization. Sapodilla is known as *chikoo* or *chiku*, in India and *sapota* in some parts of India (Tamil Nadu, Kerala, Karnataka and Andhra Pradesh) belongs to family *Sapotaceae.* The fruit was introduced during 1888 in a village Gholwad of Thane district of Maharashtra in India (Cheema., Bhatt., Naik., 1954). At present, Sapota cultivation is confined mostly to the coastal areas of Gujarat, Maharashtra and Tamilnadu (Bose., 1985). It can grow to more than 30 m (98 ft) tall with an average trunk diameter of 1.5 m (4.9 ft). The average height of cultivated specimens, however, is usually between 9 and 15 m (30 and 49 ft) with a trunk diameter not exceeding 50 cm (20 in). It is wind-resistant and the bark is rich in a white, gummy latex called chicle. The ornamental leaves are medium green and glossy. They are alternate, elliptic to ovate, 7–15 cm long, with an entire margin. The white flowers are inconspicuous and bell-like, with a six-lobed corolla. The fruit is a large ellipsoid berry, 4–8 cm in diameter, very much resembling a smooth-skinned potato and containing two to five seeds. Inside, its flesh ranges from a pale yellow to an earthy brown color with a grainy texture akin to that of a well-ripened pear (Orwa., 2009). The fruit has an exceptionally sweet, malty flavor. Many believe the flavor bears a striking resemblance to caramel or a pear candied with brown sugar. The unripe fruit is hard to the touch and contains high amounts of saponin, which has astringent properties (Morton and Julia., 1987). The trees can only survive in warm, typically tropical environments, dying easily if the temperature drops below freezing. From germination, the sapodilla tree will usually take anywhere from five to eight years to bear fruit. The sapodilla trees yield fruit twice a year, though flowering may continue year-round (Maxwell, Lewis., Betty., Maxwell., 1984). In this present investigation we study that the Chromatographic analysis of simple sugars of Banana and Chiku.

REVIEW OF LITERATURE

Thin layer Chromatography is an analytical tool which is gaining wide acceptance. The historical development, theoretical aspects and several applications have recently been discussed. (31,24,7) Although many different sorbents are available which are suitable for TLC and the majority of the workers have used silica gel with a binder for the sorbent layer. One group of compounds for which silica gel has not been a satisfactory medium is the naturally occurring free Sugars, Poor separation of some of the more common sugars and the low capacity of the plates are two disadvantages in this application (32).

Paper chromatography has proven quite useful for the separation of small quantities of many compounds. Theory, techniques and applications have been, reviewed by several authors (4, 30, 15) Linkmen's text deals strictly with the application of these techniques to investigations in plant science (19). Although the simple sugars have been separated for many years using paper chromatography, major disadvantages, are the long elution times required for satisfactory separations the associated problems of temperature fluctuation and the total time and, equipment involved if a large number of samples are to be analyzed (5).

Using cellulose as the sorbent in thin-layer chromatography should provide the advantages of paper chromatography without its disadvantages and the advantages of TLC without the problems involved with silica **gel.** Randerath has found Cellulose-TLG superior to paper in the separation of nucleotides (25). The author is aware of only one paper in the literature dealing with the separation of a few simple sugars using cellulose plates (27).

Very few efforts have been made to quantitize TLC. Only one paper has dealt with the quantitative determination of simple sugars separated by TLC (23). That procedure requires 300 to 400 ɣ of sample while the silica gel plates have a capacity of about 40ɣ

The colorimetric method of Dubois, *et al.* (12) which has found wide use and acceptance in paper chromatographic techniques is unsatisfactory for cellulose-TLC due to the persistent high background which cannot be eliminated by prewashing the plates (29). Dische has recently reviewed several colorimetric procedures which are applicable to paper chromatography (10). The reaction of enthrone with carbohydrates was first described by Dreywood (11). Since then, several

authors have reported the application of this reaction to the quantitative determination of various carbohydrates (17, 21, 22, 28). Both Koehler (17) and Bonting (8) comment on the rate of color development with fructose in comparison to that of glucose.

MATERIALS AND METHODS

Chromatographic analysis of simple sugars of Chiku

Selected samples are sliced, dried under vacuum at 60^0C for 48 h and powdered. 100.0 g of raw material was extracted with doubly distilled water 75mL, 15mL of 0.1N sulphuric acid and kept under hot plate for about 1 h at 60°C. Contents are cooled and stirred well with magnetic stirrer for 30'. Neutralized using AR barium hydroxide and precipitated barium sulphate is filtered off. The resulting syrup was stored at 4°C in the dark. The syrup was treated with charcoal (coir pith) and agitated for 30' followed by Silica gel (230-400 mesh) packed in a sintered glass crucible for about 2cm thickness connected to suction pump, where rota vapour removed the solvent of the filtrate. The residue was placed in an air tight glass container covered with 200 ml of boiling 80% ethanol. After simmering for several hours in a steam bath, the container was sealed and stored at room temperature. For the analysis, sample was homogenized in a blender for 3- 5'at high speed and then filtered through a Buchner funnel using a vacuum source replicated extraction with 80% EtOH (2 x 50mL) each time and the whole syrup was concentrated. Methanol - Dichloromethane – water (0.3:4:1, v/v/v), Sample tubes fed with the mixture were loosely capped, placed in a water bath for 5s, and left at room temperature for 10'and placed in separating funnel, agitated vigorously by occasional release of pressure, results two phases. The organic phase was discarded which removes the organic impurities and the methanol: water phase was assayed for sugar. The residues were oven-dried at 50°C overnight to remove the residual solvent, and stored at –2°C for analysis (Chandraju *et al*., 2011).

Preparation of Chromatoplates:

Thin layer chromatography was performed for the concentrated separated fraction using Cellulose MN 300 G. The fractions obtained were subjected to one dimensional chromatogram on a cellulose layer plate. Each plate was activated at 110°C prior to use for 10'.

Standard Samples:

Pure samples D(-) Arabinose, D(-) Ribose, D(+) Xylose, D (+) Galactose, D(+) Glucose, D(+) Mannose,L (-) Sorbose, D(-) Fructose, L(+) Rhamnose, D(+) Sucrose and D (+) Maltose, D(+) Lactose were used as standard.

One – Dimensional Chromatography

10 mg of each sugar and the separated fractions were dissolved in 1ml of deionised water. 1µL of each sugar solution was applied to the chromatoplate with the micropipette in the usual manner. The chromatoplate was placed in the chamber containing the developing solvent. The solvent system used was n-butanol-acetone-pyridinewater (10:10:5:5, v/v/v/v). The plates were developed in an almost vertical position at room temperature, covered with lid (Baldwin and Bell, 1955; Schweiger., 1962; Vomhot and Tucher, 1963; Lato *et al*., 1968). After the elution, plate was dried under warm air. The plate was sprayed with 5% diphenylamine in ethanol, 4% aniline in ethanol and 85% phosphoric acid (5:5:1v/v/v). The plate was heated for 10'at 105°C. While drying coloured spots appear. The Rf values relative to the solvent are reported below.

RESULTS AND DISCUSSION

Four separated and purified sample fractions are spotted in the cellulose layer and the eluted species were mentioned as F1, F2, F3, and F4 in the chromatogram shown in Fig. The fractions obtained were found to be matching with the standard sugars and found to Lactose, maltose, mannose and arabinose.

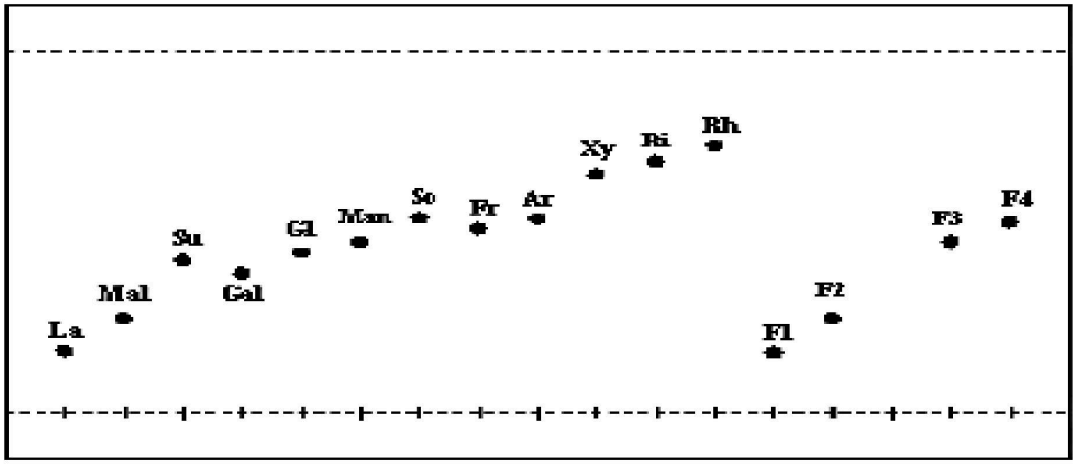

Figure 1 Developed thin layer chromatogram over a cellulose layer of Chikoo, (La – Lactose, So – Sorbose, Ar-Arabinose,
Rh – Rhamnose, Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal – Maltose).

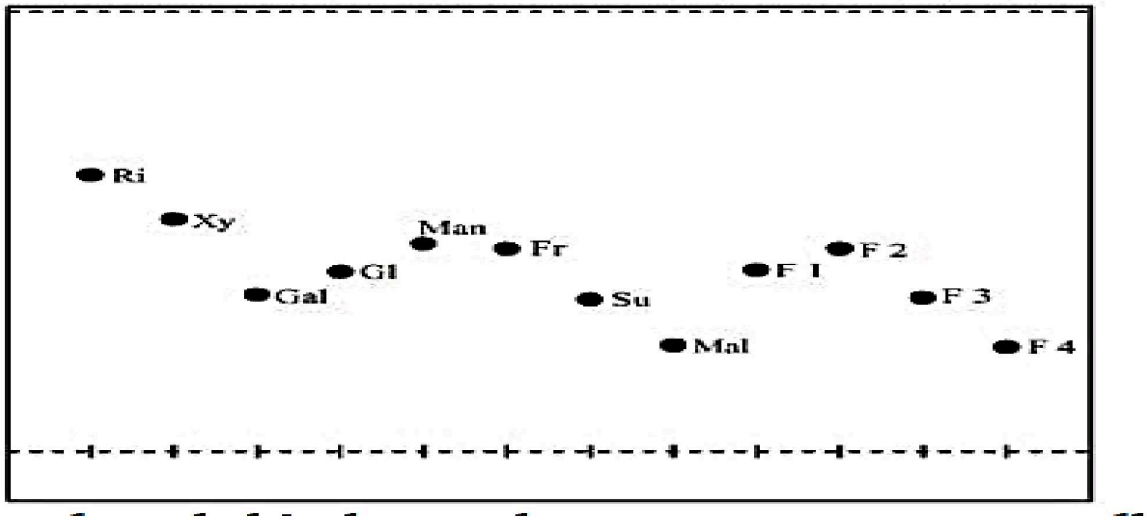

Figure 2: Developed thin layer chromatogram over a cellulose layer of Banana.

(Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal –Maltose)

CONCLUSION

The analysis of the discarded portion is very high; therefore, because of disposal problems the household solid wastes are of greater importance. A fruitful and economic industrial application was applied in this current work. Based on the above studies, a rapid method for the extraction of water-soluble sugar has been developed. The mixture MDW gives better results as compared with MCW, i.e. dichloromethane was replaced instead of chloroform (Pak *et al.,*2004). TLC analysis gives accurate confirmation for the presence of lactose, mannose, maltose and arabinose.

These household solid wastes are of greater importance because the discarded portion is very high, for instance in the present work the non- edible portion of banana which is thrown is 20%. Therefore, there is often a serious waste disposal problem. A fruitful and economic industrial application was applied in this current work. Based on the above studies, we have developed a rapid method for the extraction of water soluble sugar. TLC analysis gives accurate confirmation for the presence of glucose, fructose, sucrose and maltose. Keeping in view the results obtained, it may be concluded that banana peels possess good amount of nutritional value and rich in carbohydrates. Glass package and cold storage can retain good quality leading to more extensive shelf life of the products.

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