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Utilization of mango peels (*Mangifera indica*) for the extraction of sugars

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ABSTRACT

Mangoes have been part of human diet for ages due to their health benefits. But consumption of these fruits generates outer skin wastes that may lead to environmental pollution. This study was carried out to explore the sugar components of mango peels. Selected samples are cut into small bits, dried, powdered and were subjected to sensitive extraction procedure developed using the mixture Methanol - Dichloromethane - Water (MDW) (0.3:4:1, v) and MeOH-H_2O phase was assayed for sugar analysis. The extracted sugars were put through some chemical characterization procedures for purposes of separation and identifying its components. The various standard sugars were spotted using the solvent system n-butanol-acetone-pyridine-water (10:10:5:5, v) in the cellulose layer for TLC analysis which indicated the presence of glucose, sucrose, fructose.

Keyword: Sugar extraction; mango peels; Separation; LC/MS; TLC.

INTRODUCTION

The mango tree grows rapidly and may attain a height of 90 ft (27 m) and a spread of 120 ft (37 m). It is densely covered with glossy leaves and bears small, fragrant yellowish or reddish flowers. The fruit, a fleshy drupe, is about 6 in. (15.2 cm) long and has thick greenish to yellowish-red mottled skin, pale yellow to orange-red flesh, and a large seed, the kernel of which is edible when cooked [1]. Mango fruits are luscious, aromatic, and slightly acidic. Equivalent in importance to the apple of Europe and North America, they are a vital food source for millions of inhabitants of the tropics. Mangoes are eaten fresh (green or mature), often as a dessert fruit, and are also cooked, dried, and canned. They are used in chutneys, jellies, and jams. The tree is propagated by grafting and budding and to a lesser extent by seed [2]. Mango is a fleshy fruit containing more than 80% water [3]. Its size depends on the accumulation of water and dry matter in the various compartments during fruit growth. The skin, the flesh and the stone have specific compositions that appear to accumulate water and dry matter at different rates, depending on environmental conditions [4]. Mango dry matter mainly consists of carbohydrates, 60% of which are sugars and acids [5], the main compounds contributing to fruit sweetness and acidity [6]. Fruit flesh taste is highly dependent on the balance between organic acids and soluble sugars, which are predominantly represented in mango by citric and malic acids, and sucrose, fructose and glucose, respectively [7]. This study forms a part of a series of investigations that were carried out in our laboratory to understand sugar profiles in the peels of several fruits including pomegranate, pineapple, banana, black grape, and almond [8-18].

MATERIALS AND METHODS

Extraction

Selected samples are sliced, dried under vacuum at 60°C 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 5 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), 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.

Instrumentation

The mixture was separated in 26'by reversed phase HPLC on an Adsorbosphere column-NH_2, (250 x 4.6 mm column) using both isocratic and gradient elution with acetonitrile/water and detected using Waters ELSD 2420. In ELSD, the mobile phase is first evaporated. Solid particles remaining from the sample are then carried in the form of a mist into a cell where they are detected by a laser. The separated fractions were subjected to UV analysis using Agilent 8453 coupled with Diode array detector. HPLC–MS analysis was performed with LCMSD/Trap System (Agilent Technologies, 1200 Series) equipped with an electro spray interface. The MS spectra were acquired in positive ion mode. The mobile phase consisted of 0.10% formic acid in HPLC grade deionised water (A) (milli-q-water (subjected to IR radiation under 3.5 micron filters) and Methanol (B) taken in the stationary phase of Atlantis dc 18 column (50 x 4.6mm - 5µm). The gradient program was as follows: 10% to 95% B in 4 min, 95% B to 95% B in 1 min, 95% B to 10% B in 0.5 min followed by 10% B in 1.5 min at a flow rate of 1.2 mL min^{-1}. The column oven temperature was kept at 40°C and the injection volume was 2.0 µL. Product mass spectra were recorded in the range of m/z 150-1000. The instrumental parameters were optimized before the run.

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-pyridine-water (10:10:5:5, v). The plates were developed in an almost vertical position at room temperature, covered with lid [19-22]. 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 R_f values relative to the solvent are reported below.

RESULTS AND DISCUSSION

The MS report recorded at the appropriate time as per MSD for fraction 1 at 0.636 and 0.666 min, fraction2 at 0.578 and fraction3 at 0.593. The MS report recorded at the appropriate time as per MSD for Fraction 1 scanned between the time period 0.507:0.600min gave m/z values 126.9, 163.0, 343.2, 360.0, 365.0, 374.0 and 0.600 : 0.878 min gave

m/z values 126.9, 163.0, 342.2, 365.0, 365.0, 375.1. Fraction2 scanned between the time periods 0.493:0.772 gave m/z values 112.9, 145.1, 163.0, 164.1, 180.1, 202.9 respectively. Fraction3 scanned between the time periods 0.507: 0.745 gave m/z 111.2, 115.1, 140.9, 145.1, 180.1, 198.0, 202.9 respectively. This gives a conclusion that these masses corresponds to various monosaccharide and disaccharides FIGURE 1-3.

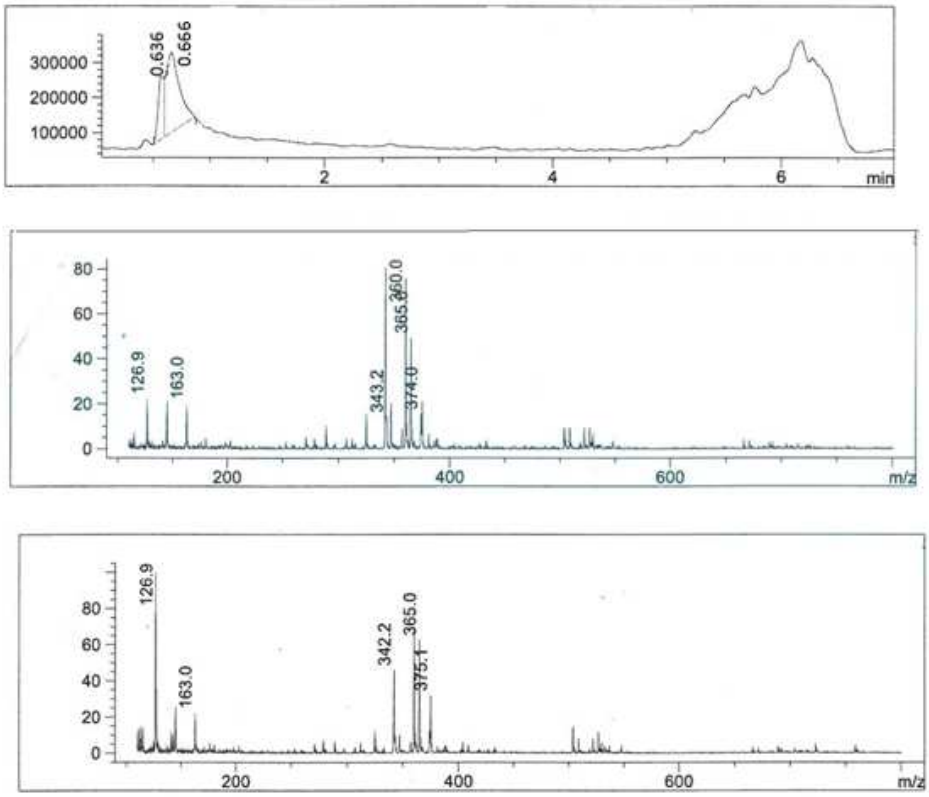

FIGURE 1: Mass report of Separated Fraction 1

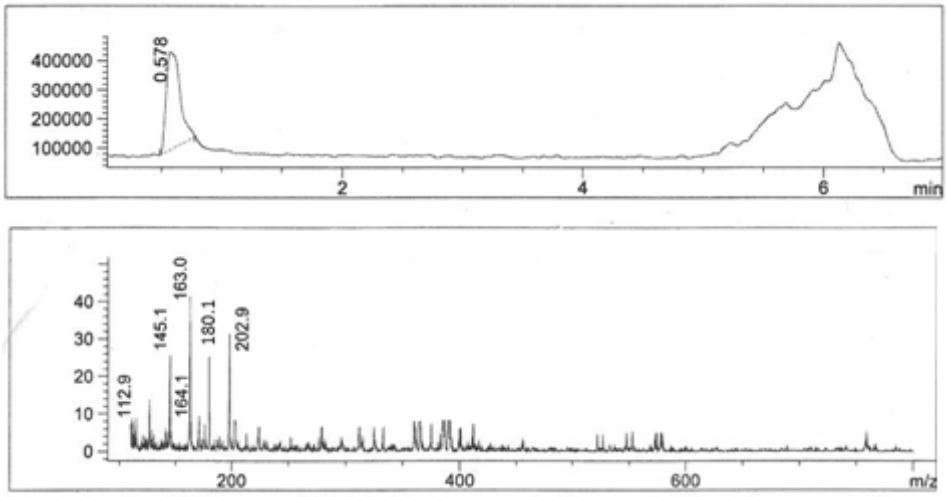

FIGURE 2: Mass report of Separated Fraction 2

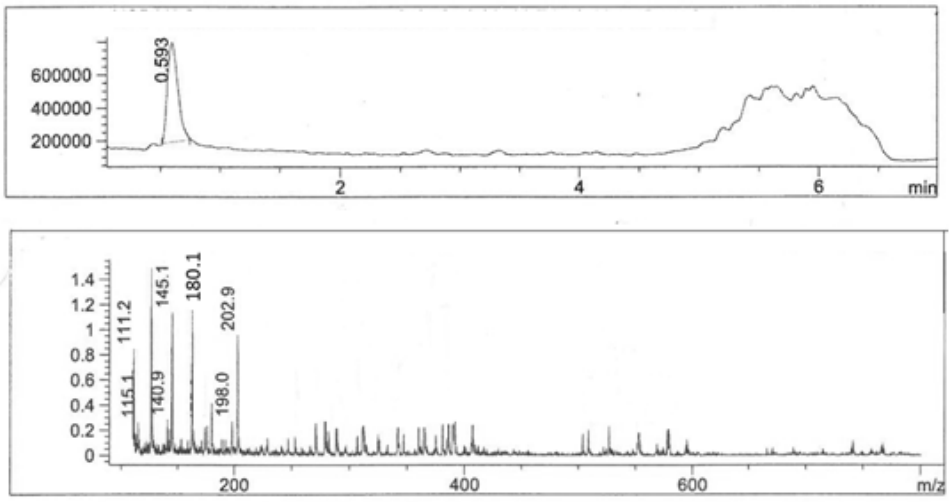

FIGURE 3: Mass report of Separated Fraction 3

Thin layer chromatographic analysis report

Three separated and purified sample fractions are spotted in the cellulose layer and the eluted species were mentioned as F1, F2 and F3 in the chromatogram shown in Fig.4. The fractions obtained were found to be matching with the standard sugars and found to sucrose, glucose and fructose. R_f value for the analytical grade samples which also shows the matching fractions TABLE 1.

TABLE1: R_f values matching of the analytical standard samples and the separated samples

Sugars	R_f (Scale of R_f =1)	Fraction matching
Lactose	0.17	-
Maltose	0.26	-
Sucrose	0.42	F1
Galactose	0.38	-
Glucose	0.44	F2
Mannose	0.47	-
Sorbose	0.54	-
Fructose	0.51	F3
Arabinose	0.53	-
Xylose	0.66	-
Ribose	0.69	-
Rhamnose	0.74	-

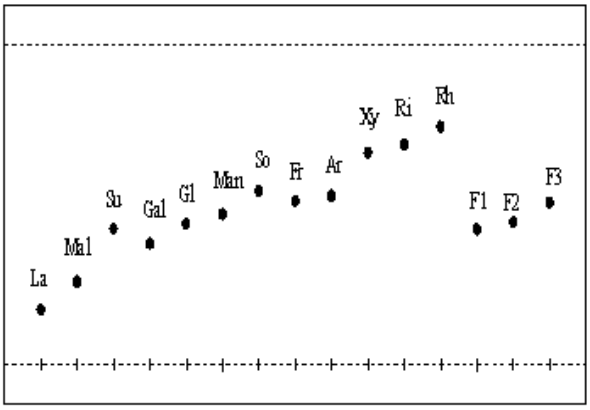

FIGURE 4: Developed thin layer chromatogram over a cellulose layer, (La – Lactose, So – Sorbose, Ar- Arabinose, Rh – Rhamnose, Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal –Maltose).

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