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ADVANCED CHROMATOGRAPHIC TECHNIQUES FOR THE ANALYSIS OF SUGARS EXTRACTED FROM SWEET LEMON PEELS

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ABSTRACT

A rapid, sensitive extraction method was developed using the mixture Methanol-Dichloromethane - Water (MDW) (0.3 : 4 : 1, v) and MeOH-H_2O phase was assayed for sugar analysis. High-performance liquid chromatography (HPLC) with Evaporative Light Scattering Detector (ELSD) coupled to electro spray ionization mass spectrometric (ESI-MS) detection in the positive ion mode gave MS and MSn fragmentation data, which were employed for their structural characterization. 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 galactose, glucose, arabinose and Xylose. This is the first assay of the sugar profile of the sweet lemon peels, which can be further developed for characterization and evaluation of their quality with regards to their sugar composition.

Key words: Sugar extraction, Sweet lemon peels, Separation, LC/MS, TLC.

INTRODUCTION

Sweet lemon peels were cut into small bits and subjected to sequential solvent system and extracted component passed through some chemical characterization procedures for purposes of identifying its sugar components. Processing of citrus peels into sugars is a sure way of transforming these wastes with great potential for environmental pollution into a resource with great potential for economic prosperity, and also for securing the public health impacts of safer and healthier environment, likely to be obtained from the indirect waste management option so offered. Mainly citrus fruit peels consist of cellulose, essential oils, proteins and some simple carbohydrates[1]. Carbohydrates are among the most abundant

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compounds in the plant world, and the analysis of sugars and sugar mixtures is of considerable importance to the food and beverage industries. Therefore production of various sugars evaluated from the non-edible portion of sweet lemon has considerable promise in the future to achieve economical profit and moreover utilization of the agro-industrial wastes for sugar production will be of immense benefit at preventing the pollution hazards associated with these wastes[2].

To our knowledge, detailed sugar studies on sweet lemon have not been published. Therefore, the objective of this research was to investigate the sugar content in sweet lemon based on liquid-liquid solvent extraction process designed. This information provides useful insights into design. 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[3-13].

EXPERIMENTAL

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 75 mL, 15 mL of 0.1 N 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 min. 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 min. followed by Silica gel (230-400 mesh) packed in a sintered glass crucible for about 2 cm 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 min. at high speed and then filtered through a Buchner funnel using a vacuum source replicated extraction with 80% EtOH (2 x 50 mL) 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 5 s, and left at room temperature for 10 min. 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 min. by reversed phase HPLC on an Adsorb sphere 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 electrospray 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 4.6 mm – 5 µm). The gradient program was as follows: 10% B 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 min.

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 1 mL of deionised water. 1 µL 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[14-17]. 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 : 1 v/v/v).

The plate was heated for 10 min. at 105°C. While drying coloured spots appear. The R_f values relative to the solvent are reported below.

RESULTS AND DISCUSSION

The mass spectrum detector gave the following spectrum of fraction1 at 0.606 and 2.637 min, fraction 2 at 0.578 min, fraction 3 at 0.595 and 2.576 min, fraction 4 at 0.584 min. The MS report recorded at the appropriate time as per MSD for Fraction1 scanned between the time period 0.507: 0.798 min gave m/z values 112.9, 145.1, 163.0, 180.1, 198.0, 360.0 and 2.495 : 2.760 min gave m/z value 112.1. Fraction 2 scanned between the time periods 0.493 : 0.772 min gave m/z values 112.9, 145.1, 163.0, 164.1, 180.1, 202.9. Fraction 3 scanned between the time periods 0.520 : 0.745 and 2.508 : 2.667 gave m/z values 111.2, 145.1, 150.1, 272.9, 305.1, 326.1, 327.1, 331.0 and 112.2, 145.1, 278.9, 312.1, respectively. Fraction 4 scanned between 0.520 : 0.745' gave m/z values 145.1, 150.1, which gives a conclusion that these masses corresponds to hexose, pentose whose masses are 180.1 and 150.1, respectively depicted in (Fig. 1-4).

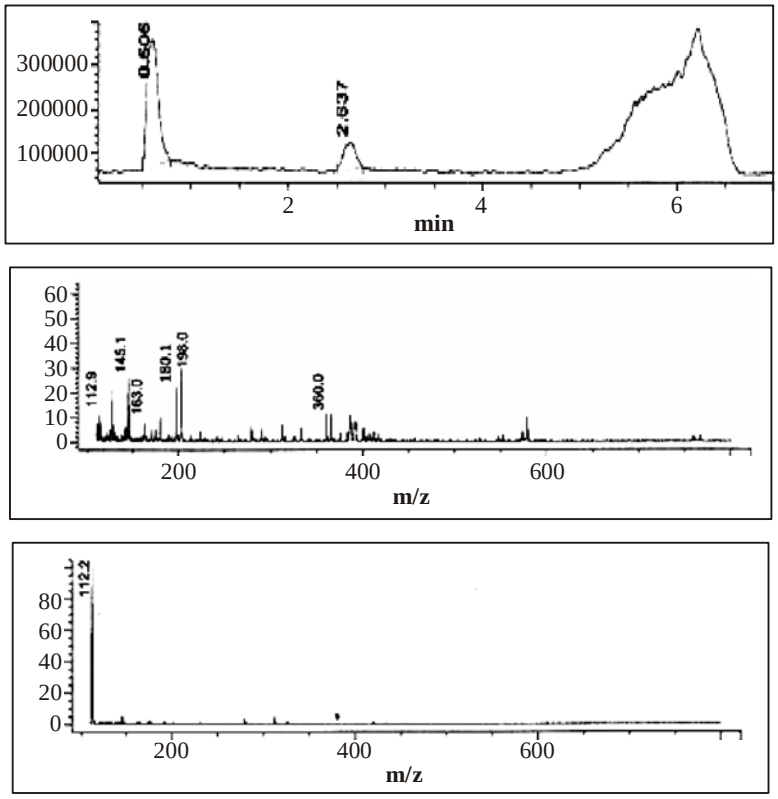

Fig. 1: Mass spectra of separated Fraction 1

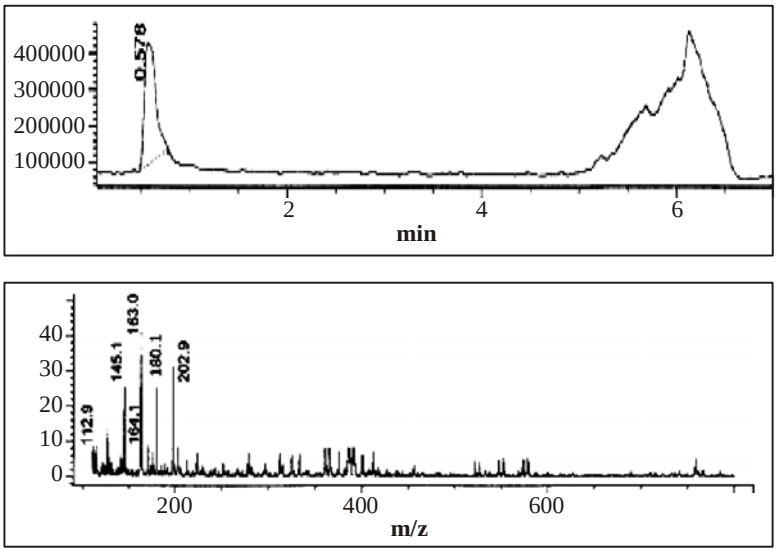

Fig. 2: Mass spectra of separated Fraction 2

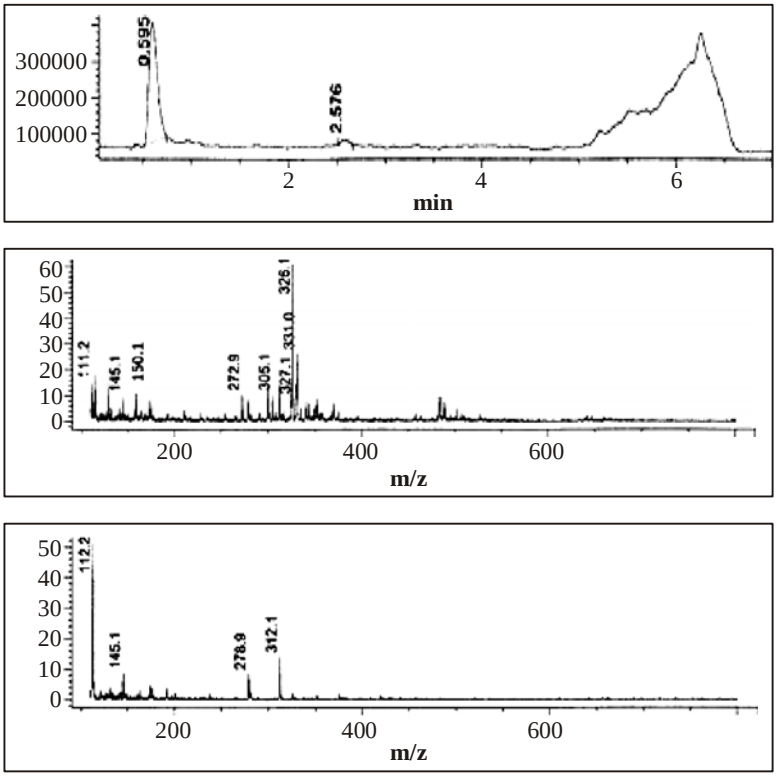

Fig. 3: Mass spectra of separated Fraction 3

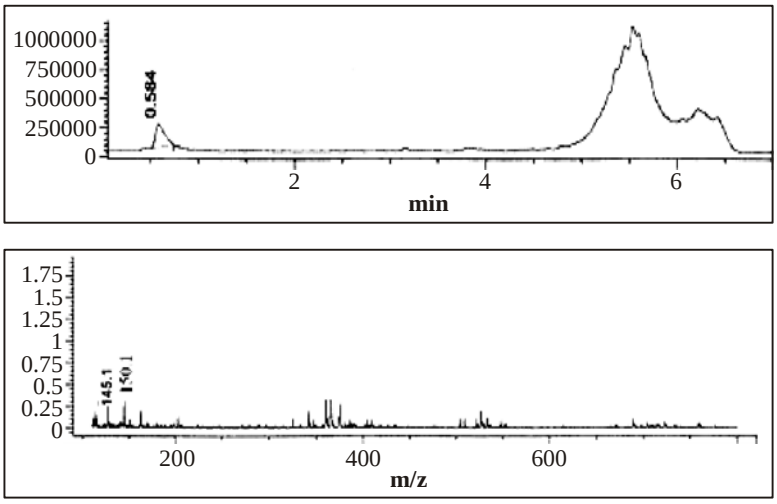

Fig. 4: Mass spectra of separated Fraction 4

Thin layer chromatographic

Analysis report

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 5). The fractions obtained were found to be matching with the standard sugars and found to Galactose, glucose, Arabinose, Xylose. R_f value for the analytical grade samples which also shows the matching fractions Table 1.

Table 1: 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	-
Galactose	0.38	F1
Glucose	0.44	F2
Mannose	0.47	-

Cont…

Sugars	R_f (Scale of R_f =1)	Fraction matching
Sorbose	0.54	-
Fructose	0.51	-
Arabinose	0.53	F3
Xylose	0.66	F4
Ribose	0.69	-
Rhamnose	0.74	-

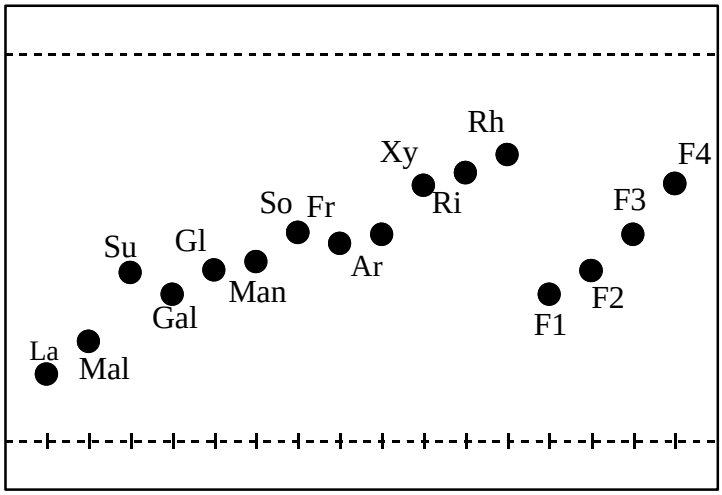

Fig. 5: Developed thin layer chromatogram over a cellulose layer, (La-Lactose, So-Sorbose, Ar-Arabinose, Rh-Rhamnose, Ri-Ribose, Xy-Xylose, Gal-Galactose, Gl-Glucose, Man-Mannose, Fr-Fructose, Su-Sucrose and Mal-Maltose)

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