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QUALITATIVE ANALYSIS OF SUGARS PRESENT IN NON-EDIBLE RIND PORTION OF MUSK MELON (*CUCUMIS MELO L.*) VIA CHROMATOGRAPHIC TECHNIQUE

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ABSTRACT

A rapid, sensitive extraction method was developed using the mixture Methanol – Dichloromethane - Water (MDW) (0.3:4:1v/v/v) and MeOH-H_2O phase was assayed for sugar analysis. Photodiode-array detection (DAD) has been used to prove the extracted compound is UV inactive, Preparative High-performance liquid chromatography (prep 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 and separation of individual components. The various standard sugars were spotted using the solvent system n-butanol - acetone - diethylamine - water (10:10:2:6, v/v/v/v) in the cellulose layer for TLC analysis which indicated the presence of lactose, sucrose and glucose. This is the first assay of the sugar profile of the non-edible portion of musk melon, which can be further developed for characterization and evaluation of their quality with regards to their sugar composition.

Keywords. Sugar extraction; Non-edible; Musk melon; UV inactive; Separation; LC/MS; TLC.

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.[1] 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.[1]

Musk melon *(Cucumis melo.L)* is one of the cheapest and most delicious fruits grown commercially in India. To the best of our knowledge, no information has been published on the agro-industrial wastes of musk melon were used for sugar production. With the aim of finding a suitable method of extracting sugars will bring immense benefit at preventing the pollutional hazards associated with these wastes and wide application in the near future in industries and most economical Process [2,3].

Experimental

Extraction

Selected samples are sliced, dried under vacuum at 60^0C for 48 hr 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 hour 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.[7-11]

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 electrospray interface. The MS spectra were acquired in positive ion mode. The mobile phase consisted of 0.10% formic acid in hplc grade deionized 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% 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.[7-11]

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 - diethylamine - water (10:10:2:6, v/v/v/v). The plates were developed in an almost vertical position at room temperature, covered with lid. [12-15] 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 above.

RESULTS AND DISCUSSION

Analysis report showed that the extracted separated components are UV inactive as in (**Fig.1**). The Mass Spectrum detector gave the following spectrum of fraction1 at 0.636 and 0.666 min, fraction2 at 0.525 and 0.702min, fraction3 at 0.578min. The MS report recorded at the appropriate time as per MSD for Fraction1 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 0.480: 0.546 min gave m/z values 115.1, 145.1, 175.9, 279.2, 312.1, 366.0, 365.0, 707.2 and 0.573: 0.812 min gave m/z values 111.2, 145.1, 279.2, 312.1, 360.0, 365.0, 707.2. Fraction3 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. Which gives a conclusion that these masses corresponds to Hexose, and disaccharides whose masses are 180.1 and 342.2 depicted in (**Fig. 2, 3, & 4**).

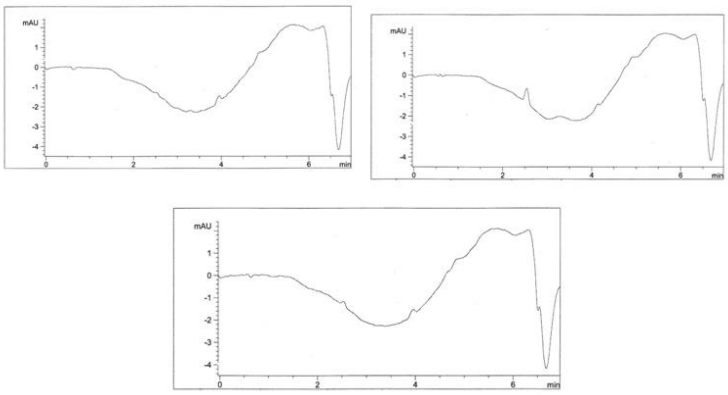

Fig.1: UV inactive spectrum of the Separated Fractions

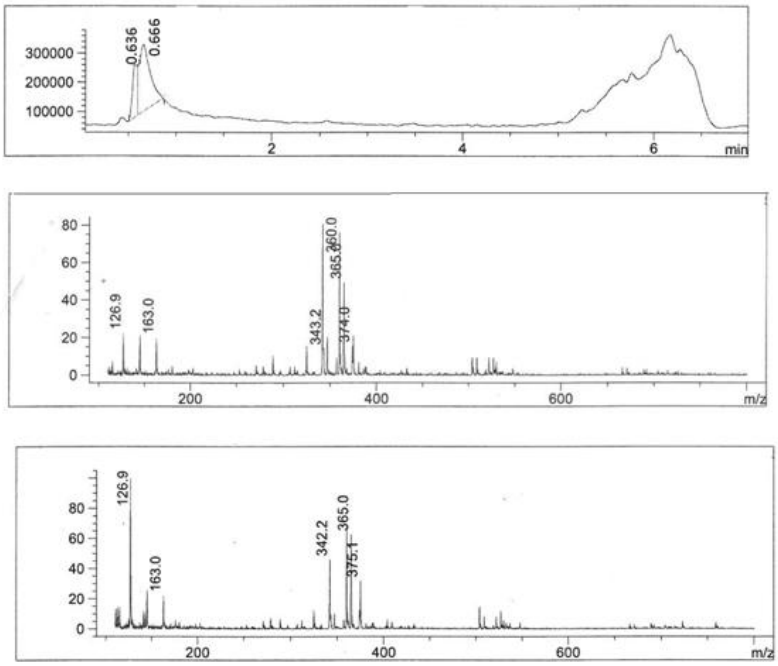

Fig. 2: Mass report of Separated Fraction

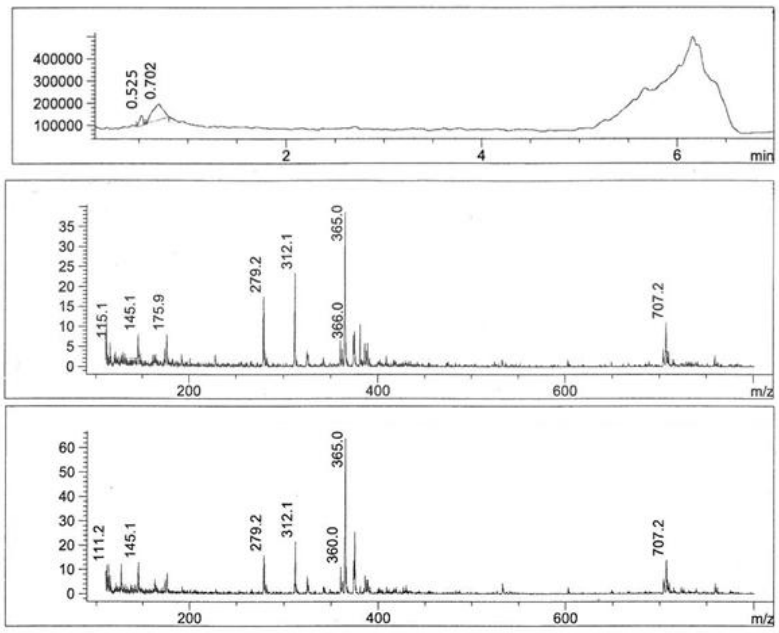

Fig. 3: Mass report of Separated Fraction 2

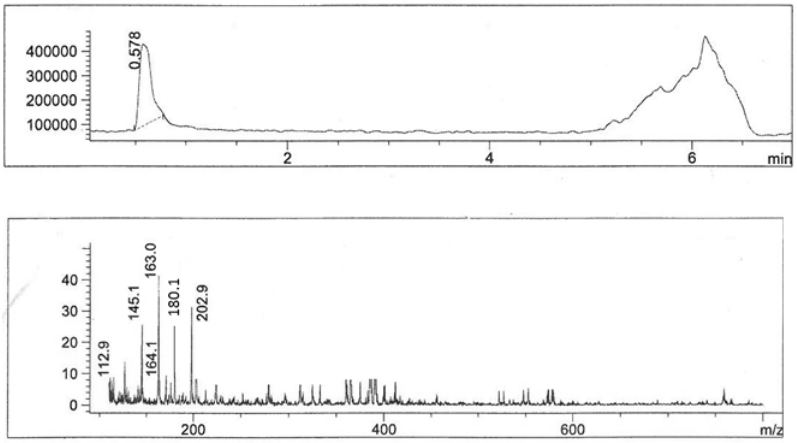

Fig.4: Mass report of Separated Fraction 3

Thin layer chromatographic analysis report

Four separated and purified sample fractions are spotted in the cellulose layer and the eluted species were mentioned as F 1, F 2 and F 3 in the chromatogram shown in (**Fig 5)**. The fractions obtained were found to be matching with three standard sugars. R_f value for the analytical grade samples shown in (**Table 1**).

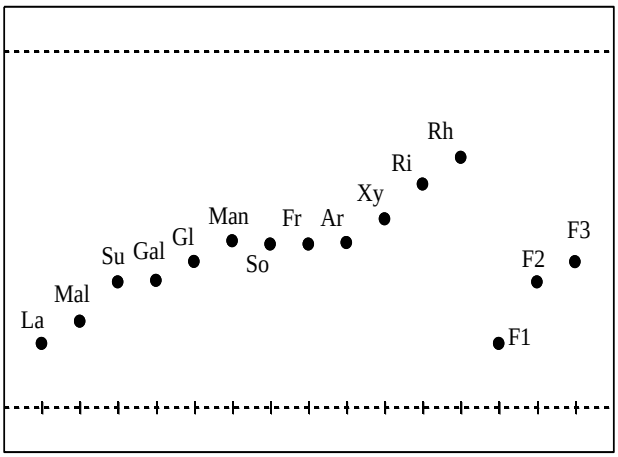

Fig. 5: Developed thin layer chromatogram over a cellulose layer, (La – Lactose, So – Sorbose, Ar- Arabinose, Rh – Rhamnose, Ri – Ribose, Xy-Xylose, Gal – Galactose, Gl - Glucose, Man – Mannose, Fr - Fructose, Su – Sucrose and Mal –Maltose).

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	**0.18**	**F1**
Maltose	**0.24**	**-**
Sucrose	**0.35**	**F2**
Galactose	**0.36**	**-**
Glucose	**0.41**	**F3**
Mannose	**0.47**	**-**
Sorbose	**0.46**	**-**
Fructose	**0.46**	**-**
Arabinose	**0.46**	**-**
Xylose	**0.53**	**-**
Ribose	**0.63**	**-**
Rhamnose	**0.70**	**-**

CONCLUSION

The quantity 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[12]. Mass and TLC analysis gives accurate confirmation for the presence of lactose, sucrose and glucose which were extracted from the outer skin of musk melon (*Cucumis melo* L.)

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