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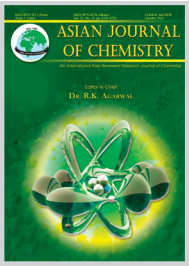

Advanced Chromatographic Technique for the Analysis of Sugars Extracted from the Peels of Black Grape (*Vitis vinifera* L.)

C.S. CHIDAN KUMAR[1], R. MYTHILY[1] and S. CHANDRAJU[2,*]

[1]Department of Chemistry, G. Madegowda Institute of Technology, Bharathi Nagar-571 422, India
[2]Department of Studies in Sugar Technology, Sir M. Vishweshwaraya Post-Graduate Center, University of Mysore, Tubinakere, Mandya-571 402, India

*Corresponding author: E-mail: chandraju1@yahoo.com

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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, high-performance liquid chromatography (HPLC) with evaporative light scattering detector (ELSD) coupled to electrospray ionization mass spectrometric (ESI-MS) detection in the positive ion mode gave MS and MSn fragmentation data, which were employed for their structural characterization. Various standard sugars were spotted using the solvent system *n*-propanol-ethyl acetate-water (13:37:7, v/v/v) in the cellulose layer for TLC analysis which indicated the presence of aldohexose glucose and ketohexose fructose. This is the first assay of the sugar profile of the black grape peels, which can be further developed for characterization and evaluation of their quality with regards to their sugar composition.

Key Words: Black grape peel, UV-Inactive, Prep HPLC/ELSD/ESI-MS, TLC.

INTRODUCTION

Household solid wastes are of greater importance because the discarded non-edible portion is very high in the case of various fruits and vegetables. Therefore, there is often a serious waste disposal problem, which can be overcome by adopting a fruitful and economic industrial application employing the extraction procedure. Sugars, quantitatively the largest organic compound group on earth, are widely distributed among both flora and fauna. Higher classes of vegetation and algae contain large quantities of sugars and the shells of arthropods, represented by crabs and shrimp, are made of chitin, which are polysaccharides. Although sugars represent a huge biomass. They also exist in very small amounts within individual living organisms. Various kinds of sugars and compound sugars are involved in bodily functions and as sources of energy. Sugars are used as raw materials within the textile, food processing and pharmaceutical industries. In the current work fruit under investigation was black grape peels.

Grape (*Vitis vinifera* L.) is one of the world's largest fruit crops[1]. World production of grape was 67 million metric tonnes from 7.3 million hectares in 2007. Grape contains the solid waste with extreme waste production as high as 20 % leftover generally consists of skins and seeds. One product produced from grape waste is the grape seed oil, which has the many nutritional properties like cholesterol free, low in saturated fats, contains linoleic acid and high-density lipoprotein and rich in vitamin E and antioxidants[2]. Polyphenol compounds are great importance for the food and drink products derived from plant, since these compounds are responsible for their organoleptic properties, makes their analysis of considerable interest. The consumption of foods rich in polyphenols helps in the prevention of cardiovascular diseases, certain types of cancer and other diseases related to aging, because of their antioxidant properties. The great advantage of wine as a matrix for polyphenols in the diet is that in wines they are present in the soluble state and are hence more biologically available, in contrast with plant foods that contain their polyphenol compounds in polymeric, insoluble or strongly bonded forms and are thus less available for absorption. Most of the past research dealing with the analysis of polyphenols has traditionally used high-performance liquid chromatography (HPLC) coupled with liquid chromatography-mass spectrometry (LC-MS) was being used for this purpose[3]. The present investigation is concentrated mainly on the water soluble sugars present in the peels of black grapes which have wide application in industries and most economical process.

EXPERIMENTAL

Extraction: Selected samples are sliced, dried under vacuum at 60 ºC for 48 h and powdered. 100 g of raw material was extracted with doubly distilled water 75 mL and stirred well with magnetic stirrer for 0.5 h. The resulting syrup was stored at 4 ºC in the dark. The syrup was treated with charcoal (coir pith) and agitated for 0.5 h 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 × 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 min 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 ºC overnight to remove the residual solvent and stored at -2 ºC for analysis[4,5].

Instrumentation: The mixture was separated by reversed phase HPLC on an adsorbosphere column-NH_2, (250 × 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. Crude sample and 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 mass 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 × 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 ºC 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[6-9].

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.

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.

One-dimensional chromatography: 10 mg of each sugar and the separated fractions were dissolved in 1 mL of deionized 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*-propanol-ethyl acetate-water (13:37:7 v/v/v). The plates were developed in an almost vertical position at room temperature, covered with lid[10-12]. After the elution, plate was dried under warm air. The plate was sprayed with 3 % *p*-anisidine hydrochloride in *n*-butanol and heated for 2 to 10 min at 100 ºC. While drying green-brown, yellow coloured spot appeared corresponding to aldohexose and ketohexose respectively[13]. The R_f values relative to the solvent are reported below.

RESULTS AND DISCUSSION

Analysis report showed that the crude sample is UV-Inactive Fig. 1. The retention time of HPLC for crude gave a merged single ionized peak at the R_t = 0.637 and separated fractions 1 & 2 at the R_t = 0.662, 0.676 are mentioned under Figs. 2 and 3 respectively. The mass spectrum detector (MSD) scanned the mass ionization peak for the crude mixture at 0.434 and 0.572 min, fraction1 at 0.578 min, fraction 2 at 0.593 min respectively. The mass spectra report recorded at the appropriate time as per MSD for crude mixture between time period 0.387:0.493 and 0.520: 0.758 gave the m/z 115.1, 140.9, 143.1, 160.9, 173.1, 199.1 and 126.9, 163.0, 202.9 gives the conclusion that the sugars may be hexose monohydrate with mass 199.1 in the positive mode and their fragments. Fraction 1 scanned between the time period 0.493:0.772 gave m/z 112.9, 145.1, 163.0, 164.1, 180.1, 202.9, which gives a conclusion that it could be a hexose *i.e.*, glucose, fructose, galactose, mannose *etc*., where mass corresponds to 180.1 shown in Fig. 4. Fraction 2 scanned between the periods of 0.507: 0.745 gave m/z 111.2, 115.1, 140.9, 145.1, 180.1, 198.0, 202.9, respectively which again gives the same conclusion as narrated above (Fig. 5). Two separated and purified sample fractions are spotted in the cellulose layer and the eluted species were mentioned as F1, F2 in the chromatogram was depicted in Fig. 6. R_f value for the analytical grade standard samples were found to be matching with glucose and fructose and the values are tabulated (Table-1).

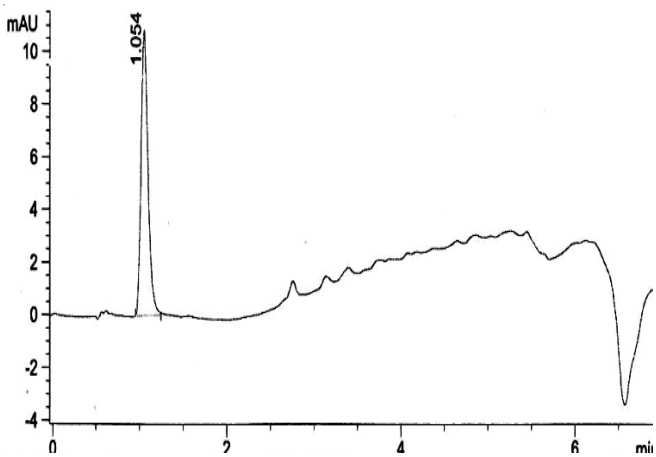

Fig. 1. UV Report-crude mixture

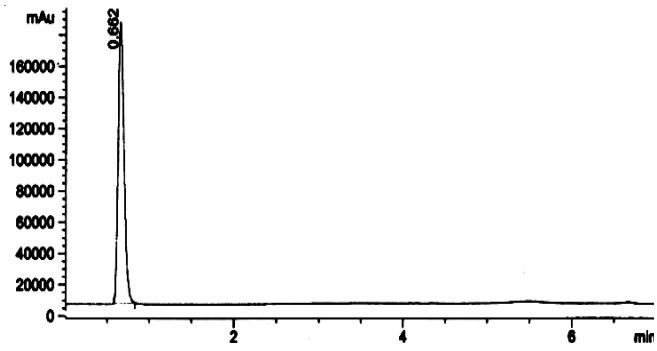

Fig. 2. HPLC report-fraction 1

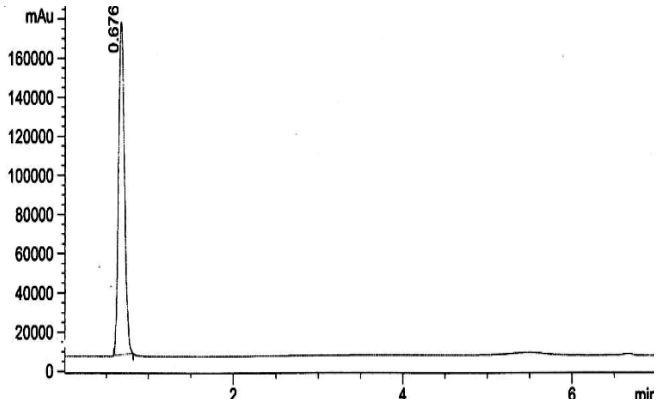

Fig. 3. HPLC report-fraction 2

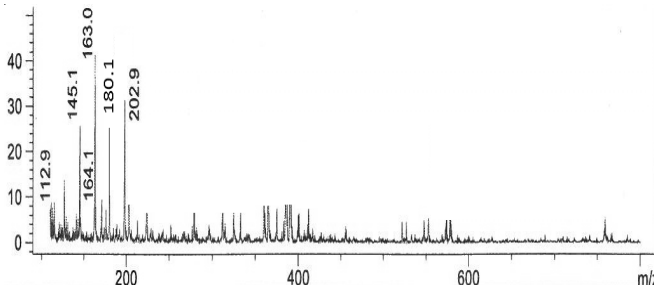

Fig. 4. MS report for the separated fraction 1 (Time 0.493:0.772)

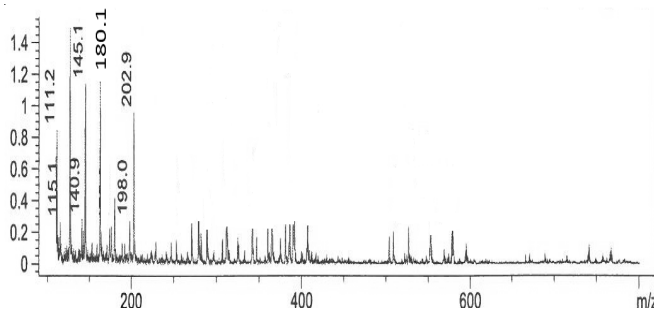

Fig. 5. MS report for the separated fraction 2 (Time 0.507: 0.745)

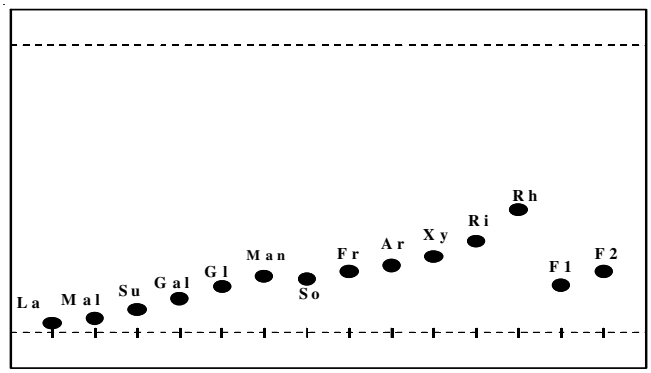

Fig. 6. Developed thin layer chromatogram over a cellulose layer, (La - lactose, So-sorbose, Ar-arabinose, Rh-rhamnose, Ri-ribose, Xy-xylose, Gal-galactose, Gl-glucose, Man-mannose, Fr-fructose, Su-sucrose and Mal-maltose)

Conclusion

A fruitful and economic industrial application was applied in this current work. Based on the above studies, a rapid method for the extraction and analysis of water soluble sugar has been developed. The mixture of methanol- dichloromethane-water gives better results as compared with methanol-chloroform-water[14]. HPLC has proven to be more selective than conventional wet methods; additionally, HPLC allows individual quantification of several individual sugars in a single chromatographic run. Mass and TLC analysis gives accurate confirmation for the presence of glucose and fructose which were extracted from the peels of black grape.

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