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HPLC analysis and determination of saccharides in selected fruit and vegetable juice

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Abstract- High performance liquid chromatography (HPLC) method for the determination of main three sugars (glucose, sucrose and fructose) individually by chromatography to identify and quantify the amount of mentioned sugars. The determination of sugar is important for quality and assurance of fruits and vegetables. The present work reports the sugar analysis of extracted apple juice, apple pulped juice, carrot juice and cabbage juice using HPLC method, the sugars (glucose, sucrose and fructose) were separately analysed and quantified using different revers phase methods. The higher amount of sugars were observed in pulped apple juice but the cabbage juice was contain lesser amount of sucrose and the lesser amount of fructose was seen in carrot juice.

Key words: HPLC, glucose; sucrose; fructose; juice;

1. INTRODUCTION

Fruit juice is consider one of the healthiness food in human diet, the prepared juice to maintain their nutritional effects, sugars are the main component found in all beverages, they present naturally and additives to express sweetness and texture. Sugars are also used as preservatives, sucrose, glucose and fructose are the main sugar found in fruit juices. Organic acids are another important component of fruit juices which determine the tartness and flavour of juice such as malic acid and citric acid [13]. There has been the increasing for consumption and production of juices in both urban and rural area of the word. Pure fruit juice main accuracy requests those caused from employing less price and low quality ingredients, instead of those declared on the product labels. The main component of juices is water and carbohydrates such as glucose, sucrose and fructose [12]. Different types of fruits and vegetables are processed to juice, due to the loss of some nutrients during processing of juice some cheaper juices, acids, sugar, water and other component are added to the pure juice. The variation of ingredients specially composition of sugar, vitamins and organic acids are influenced the taste and health benefit of juices [11].

Juice adulteration is done by adding cheaper components mainly combination of sugar solutions and syrups by mixing of cheaper with expensive juices, another most important method for juice adulteration is using inexpensive commercially accessible sweeteners such as high fructose corn syrup (HFCS), hydrolysed inulin syrup, and inverted beet/cane sugar. By addition of carbohydrates can maintain the expected taste and brix value [5] Several methods have been published for estimation of

sugars. High pressure liquid chromatography (HPLC) is an important method to determine the quantification of carbohydrate, the determination of carbohydrates in fruit juices are not only necessary for establishing the accuracy but also important for assessing the quality and checking the possible microbiological changes during storage [5, 3] Determination of individual sugars in fresh fruits, vegetables and their juices products are the important analysis of food nutrients, it means to evaluate the quality, contamination and finding of adulteration [8]). Many conventional methods for determination of sugar concentration in food ore based of refractive index method which give information regarding the amount of dry matter contain of juice and provide a quick evaluation about total sugar. Also different volumetric procedures provide information about total sugar content and the amount of glucose and fructose [14] HPLC is the powerful method to analysis of carbohydrates, organic acids, preservatives and different other ingredients in food samples [7] it is the faster and easier technique to analysis the sugar and acids in foods and beverages [4].

2. MATERIAL AND METHODS

1.2. Samples

The matured ripen fruit and vegetable (apple, carrot and cabbage) were purchased from super market, samples were chosen by the study as they are usually consumed by common people and used as main ingredients in many diet, the samples were selected with uniformity of colour, size and freedom from blemishes.

2.2 Chromatographic apparatus

A HPLC equipped with a pump delta chrom^{TM} system (SDS030), a refractive index detector (R1830), analysis, RI detector (K/2301) (KNAUER Germany), the micro filter ProFill 25µm HPLC syringe filter nylon (PA). ultrasonic bath type UC 002BM!, (Telsa Stropkov, Slovak republic). Column Nucleodur 100-5 NH_2- RP (250 x 4µm) (Duren. Germany) and the controller evaluation were with program clarity version 2.4.1.56.

3.2 Liquid chromatographic method

The liquid chromatographic method used for the determination of saccharides (glucose, fructose and sucrose) was done using phenomenex NH_2 column and acetonitrite water (75:25 v/v) as mobile phase. The analysis was carried out in isocratic mode at flow rate of 1ml/min, a refractive index detector was used for the separation of sugars, and column oven temperature was maintained at 40^{o}C.

All the solutions were filtered trough a 0.5µm nylon membrane filter before HPLC analysis and the mobile phase solvent were degased before use, for all the components the conditions like equilibration and mobile phase flow rate were standardized according to the method used for best results before subjecting the samples to the process.

4.2 Standard solution preparation

The standard solution of sugar was prepared in volumetric flask by dissolving of required amount of reagent in 50ml of Millipore water. The standard concentration range chosen 0.05-2.05% for glucose, fructose and sucrose, each standard solution was subjected to chromatographic run and their chromatograms were recorded.

5.2 Analysis of extracted juices

Juices samples were analysed under the same conditions as standards, chromatographic peaks were identified by comparing retention times of separated components in chromatograms of juice samples with pure compounds. Data and all calculation were done by Shmadzu LC solution software, the result of calculation represented by percentage of individual sugars of various juices. Quantification values were obtained by applying their peak areas to the calibration curves. Three different samples of same juice were analysed, good repeatability was obtained in terms of retention times of different constituents under study, for quantification, the values were averaged for the three values obtained for each juice.

6.2 Sample preparation

The good quality samples were cleaned washed, peeled, after removing the skins noted down the weight. Then the juice extracted by Moulinex juicer, the extracted juice was filtered with 0.5μm nylon (PA) membrane and removed all sample impurities for further analysis.

3. Result and discussion

To determine the quantity of the specific sugar concentration in a sample, several modern instrumental methods can be used including enzymatic analysis, chromatographic methods and electrochemical of spectrometric methods [1, 11] The chromatographic techniques are very accurate and they are reduce the time consuming and require extensive sample preparation [9].

1.3 Optimization of chromatographic method

Initially the chromatographic conditions such as mobile phase composition, flow rate and column temperature were optimized for separation of individual sugars.

2.3 Chromatographic performance

Peak identification based on the retention time (t_R.) Identification of the three sugars were confirmed with known standard injected separately through the HPLC and the retention time for apple juice sugars (glucose, sucrose and fructose) were (t_R=7.69 minutes,, t_R=10.74 minutes and t_R=6.82 minutes). The retention time for cabbage juice sugars (glucose, sucrose and fructose) were (t_R=7.61 minutes. t_R=10.64 minute and t_R=6.77 minutes), the retention time for carrot juice sugars (glucose, sucrose and fructose) were (t_R=7.64 minutes, t_R=10.69 minute and t_R=6.81 minutes), and the retention time for pulped apple juice sugars were (glucose t_R=7.57 minutes, sucrose t_R=10.69 minutes and fructose t_R=6.70 minutes). The flow rate for 50mg/L was 1ml/min, the column temperature was 40^{o}C and the injection volume 5μl (figures 1, 2 and 3) shows the typical overlay chromatograms for standard of glucose, sucrose and fructose which were injected separately to confirm the proper check for the retention time as well as presented the calibration curve for the tree separate sugars, it is shows very good reproducibility of the retention time, nice response for the peak area, excellent linearity and it is covers the concentration of apple sample, cabbage and carrot juice with pulped apple juice. In the chromatographic analysis with sugar (figures 4, 5, 6 and 7) shows a typical chromatographic separation patent of the standard of three sugars under study (glucose, sucrose and fructose). After the optimization of the conditions were carried out for the best signal, best stability and separation. The chromatographic profiles showed very good separation of the three sugars and regarding the

calibration, the liner range was on average from 0.5 to 50mg/ml for all sugars with correlation factors of 0.9995 to 0.9998. Tables 1, 2 and 3 show the peak area for individual sugars which shows the linear relationship with the concentration of related sugar and the table 4 indicates the concentration of saccharides and the peak area of evaluated samples,

Table-1: concentration values of peak area for glucose

c ($mg.cm^{-3}$	Peak area (mg/s)
10.01	1832.018
5.005	937.318
4.004	762.605
3.003	563.516
2.002	366.346
1.001	185.852

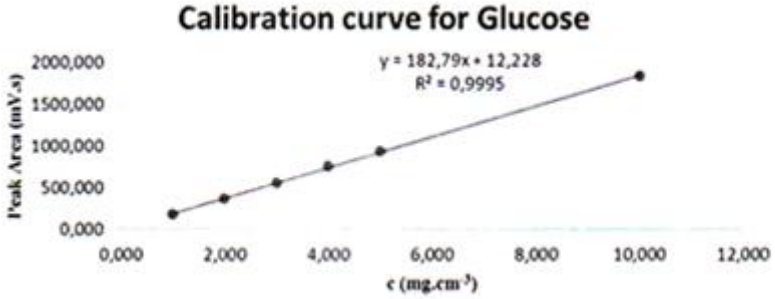

Figure-1: glucose calibration verve

Table-2: concentration values of peak area for sucrose.

c ($mg.cm^{-3}$	Peak area (mg/s)
20.052	3915.601
10.026	1990.242
8.021	1613.530
6.016	1194.384
4.010	782.029
2.005	398.254

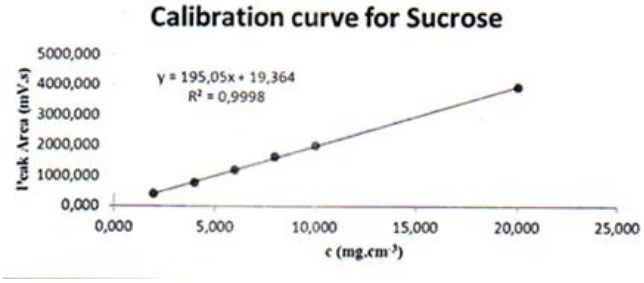

Figure-2: sucrose calibration verve

Table-3: concentration values of peak area for fructose.

c ($mg.cm^{-3}$)	Peak area (mg/s)
10.148	1916.663
5.074	973.030
4.059	790.046
3.044	584.482
2.030	379.322
1.015	198.152

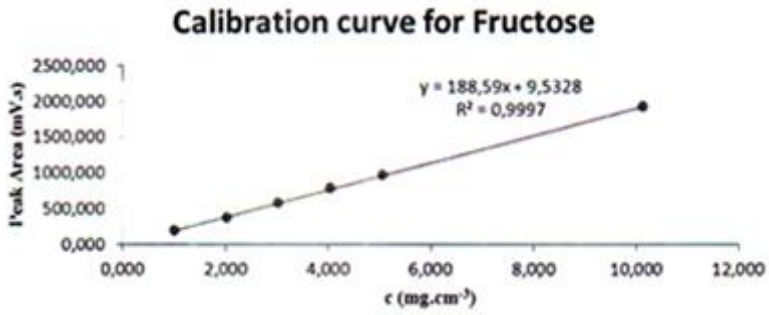

Figure-3: fructose calibration verve

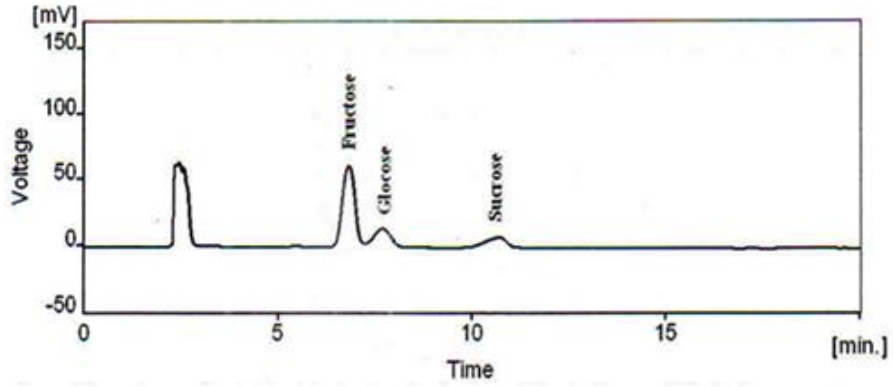

Figure-4: Chromatogram of apple juice (retention times for fructose = 6.82min, glucose = 7.69min, sucrose = 10.74 min)

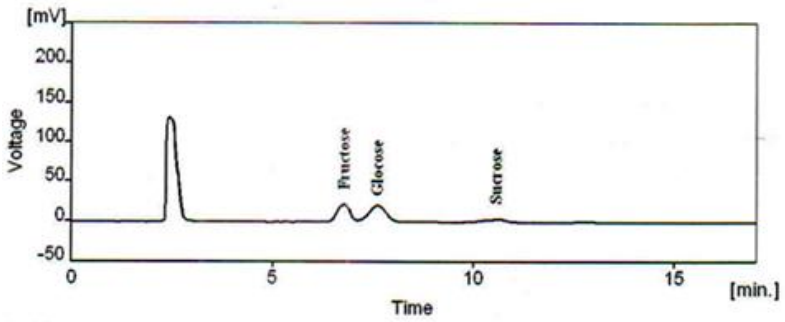

Figure-5: Chromatogram of cabbage juice (retention times for fructose = 6.77min, glucose = 7.61min, sucrose = 10.64 min)

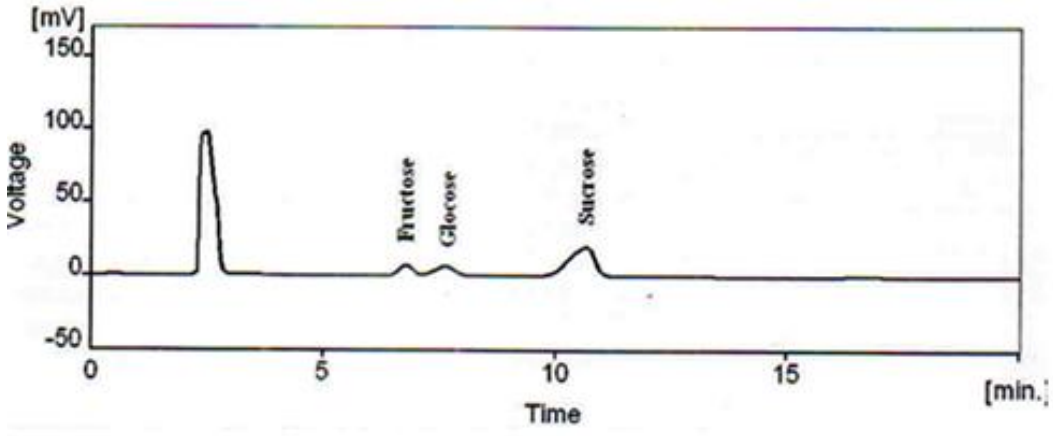

Figure-6: Chromatogram of carrot juice (retention times for fructose = 6.81min, glucose = 7.64min, sucrose = 10.69 min)

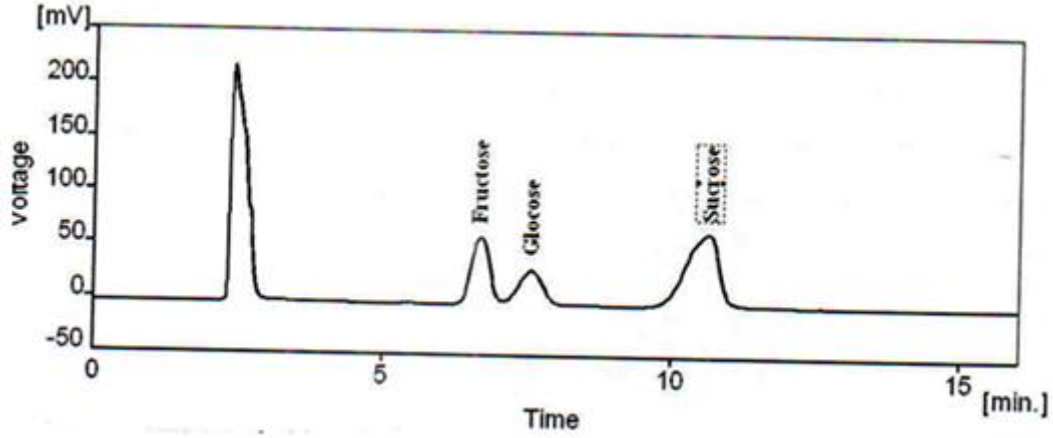

Figure-7: Chromatogram of pulped apple juice (retention times for fructose = 6.70min, glucose = 7.57min, sucrose = 10.64 min)

Table-4: Peak area and saccharides concentration in the samples

Sample peak area	Fructose peak area	Glucose peak area	Sucrose peak area	c Fructose (g100.cm-3)	c Glucose (g.100cm-3)	c Sucrose (g.100cm-3)
Cabbage juice	507.1	662.12	156.26	2.74	3.69	0.9
Apple juice	1423.65	486.5065	336.39	7.6	2.73	1.82
Carrot juice	159.77	228.58	817.75	0.9	1.32	4.29
Pulped apple juice	1380.85	994.75	4227.51	7.37	5.51	10.27

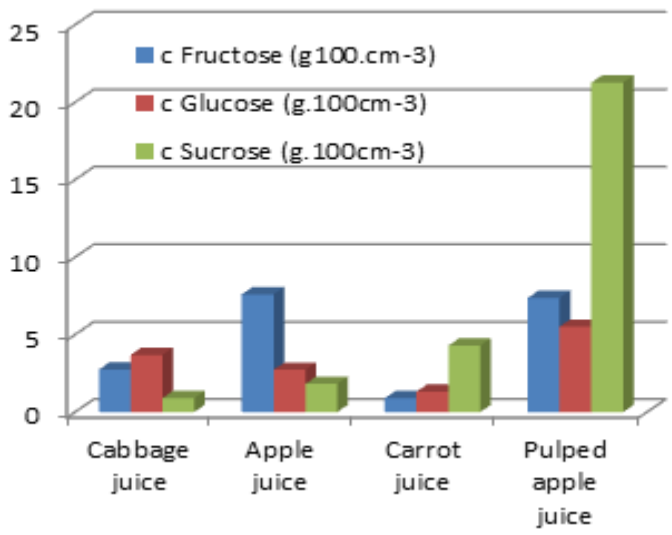

Figure-7: Saccharides content in the samples

4. Conclusion

The method used in this research for estimation of sugars (glucose, sucrose and fructose) were simple suitable and rapid procedure of HPLC method, glucose, sucrose and fructose markers of selected fruit and vegetable juice can be efficiently analysed, compared and quantified by presented RP- HPLC methods. The results obtained in term of retention times of sugars and agree well with earlier reported work (Kqradeniz 2003, Sanchez-mata Maria et al.2002, Ganjan Tyagi et al. 2011). Table-4 indicates the peak area and sugars content of analysed samples, the apple juice and pulped were content of high amount of fructose (7.60 and 7.37 g/100cm^{-3}) also the apple puled had the highest amount of sucrose and glucose (21.77 and 5.51 g/100cm^{-3}). But the cabbage juice was minimum in sucrose concentration (0.90g/100cm^{-3}).

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