Determination of Sugar Content in Fruit Juices Using High Performance Liquid Chromatography

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Abstract

Sugar is a sweet, water-soluble, crystallizable material which is obtained commercially from sugarcane or sugar beet. As an important source of dietary carbohydrate, a sweetener, preservative in foods and a cause factor for Diabetic disease, analysis of sugar content is needed. The objective of this study was to verify the suitability and determination of sugar content in fruit juices using HPLC. The fresh fruits except strawberries were peeled, cut and blended using homogenizer. After filtration, 12.5mL of each fresh juices and packed juices were diluted with acetonitrile and distilled water (50:50). The diluted fresh juices and packed juices were loaded onto C_{18} Sep-Pak cartridge. Fructose, glucose, and sucrose of each fresh juices and packed juices were analyzed in HPLC with Refractive Index detector, NH_2 polar bonded phase column, 10µm (250mm × 4.6mm I.D.), temperature of 43.5°C, mobile phase of acetonitrile and 10mM sodium phosphate (monobasic) (78:22) and flow rate of 1.0mL/min. The method validation showed good linearity of equations for fructose, glucose and sucrose were y = 3833208.4806x – 94721.0361, y = 3782886.4708x – 101683.4708, y = 3770593.9638x – 82870.9083 with regression coefficients, r^2 of 0.9995, 0.9997 and 0.9996 respectively. The calculated regression function coefficients (Vx0) for fructose, glucose and sucrose were 0.0152, 0.0112 and 0.0132% respectively. The percentage recovery of fructose, glucose, and sucrose was found to be in a range of each 86.681 – 89.888, 86.898 – 90.029, and 94.541 – 97.885%. Sugar contents in fruit juices can be determined using verified HPLC method with Refractive Index Detektor. Fructose, glucose and sucrose of fresh juices contained 0.469 – 1.431, 0.454 – 1.286, 0.544 – 1.861%, whereas that of packed juices contained 0.309 – 1.587, 0.261 – 0.762, 0.063 – 0.898%, respectively.

Keywords: Fructose, glucose, sucrose, fruit juice, HPLC.

Abstrak

Gula merupakan zat yang berasa manis, larut dalam air, berbentuk kristal yang dapat diperoleh dari berbagai sumber antara lain bit gula dan tebu. Sebagai sumber karbohidrat, pemanis dan pengawet dari makanan, dan penyebab diabetes, maka kandungan gula perlu ditentukan. Tujuan dari penelitian ini adalah untuk verifikasi kesesuaian penentuan kadar gula dalam jus buah menggunakan Kromatografi Cair Kinerja Tinggi (KCKT). Buah segar kecuali stroberi dikupas kemudian dipotong dan dicampur dengan menggunakan homogenizer. Setelah disaring, 12.5mL jus segar dan 12.5mL jus kemasan diencerkan dengan asetonitril dan air suling (50:50). Jus segar dan jus kemasan kemudian dilewatkan melalui tabung C18 Sep-Pak. Fruktosa, glukosa dan sukrosa kemudian dianalisis menggunakan KCKT detektor Indeks bias, kolom NH_2, 10µm (250µm × ID 4.6mm) dan suhu kolom 43.5ºC, fase gerak asetonitril dan larutan natrium fosfat 10mM (78:22) dan laju aliran adalah 1.0mL/min. Metode validasi menunjukkan linearitas persamaan yang baik untuk fruktosa, glukosa dan sukrosa yaitu y = 3833208,4806x – 94721,0361, y = 3782886,4708x – 101683,4708, y = 3770593,9638x – 82870,9083 dengan koefisien korelasi, r^2 adalah 0,9995, 0,9997 and 0,9996 masing-masing. Koefisien fungsi regresi yang dihitung (Vx0) untuk fruktosa, glukosa dan sukrosa adalah 0,0152, 0,0112 dan 0,0132 %. Persentase perolehan kembali untuk fruktose, glukosa dan sukrosa berada dalam rentang masing-masing 86,681 – 89,888, 86,898 – 90,029, dan 94,541 – 97,885%. Kandungan fruktosa, glukosa dan sukrosa dapat ditentukan dengan menggunakan kromatografi cair kinerja tinggi dengan detektor indek bias. Fruktosa, glukosa dan sukrosa jus segar mengandung 0,469 – 1,431, 0,454 – 1,286, 0,544 – 1,861% sementara pada jus kemasan mengandung masing-masing 0,309 – 1,587, 0,261 – 0,762, 0,063 – 0,898%.

Kata kunci: Fruktosa, glukosa, sukrosa, jus buah, HPLC.

Introduction

Refering to the Best of Health by Sheldon Zerden, it showed that a rise in sugar consumption lead to diabetes mellitus. Diabetes mellitus has been increasingly rapidly along with increases in obesity and cardiovascular diseases. In 1985, it is estimated that approximately 30 million people worldwide had diabetes. A decade later, this estimate had risen to 135 million. In 2000, this estimate had risen to 171 million. This is projected to increase to at least 366 million by the year of 2030.

In 2009, American Heart Association (AHA) released new guidelines limiting the amount of added sugar considered acceptable for a healthy diet. It should consume no more than 100 calories of added sugar for women and no more than 150 calories of added sugar

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for men. These numbers average out to about 6-9 teaspoons, or 25-37.5g, of sugar a day. Preschoolers with a daily caloric intake of 1200-1400 calories should not consume more than 170 calories, or about 4 teaspoons, of added sugar a day. Children ages 4-8 with a daily intake of 1600 calories should consume no more than 130 calories, or about 3 teaspoons, of added sugar a day. As the child grows into his pre-teen and teen years, and his caloric range increases to 1800-2000 a day, the maximum added sugar in his daily diet should be 5-8 teaspoons (Sheldon 2004; Bazzano 2004).

According to Food and Drug Administration (FDA) in 1993, sugar analysis on monosaccharides and disaccharides is a necessity for all of the food industry. Previous reports on sugar analysis of food products were based on total sugar or sucrose, with little emphasis on the other individual sugars (Ferrier 2003).

Recently, high performance liquid chromatography has become the preferred methods for quantitating simple sugars in a variety of food products (Perez *et al.* 1997; Graves and Peckham, 1995; Angustin and Khor 1986; Ellefson 2002).

Therefore, the aim of the study was to verify the suitability of determination of sugar contents in fruits using High Performance Liquid Chromatography (HPLC).

Experiment

Materials

Glucose (Merck), fructose (Merck), sucrose (Merck), acetonitrile HPLC grade (J.T Beaker), distilled water, 10mM sodium phosphate (monobasic) solution, fruits (apple, guava, kiwi, orange and strawberry), packed fruit juices (apple juice, guava juice, kiwi& grape juice, orange juice and strawberry juice).

Equipments

Weighing paper, weighing machine, spatula, volumetric flask (10mL, 25mL, 100mL), funnel, glass rod, measuring cylinder (10mL, 100mL), beaker (50mL, 100mL, 500mL), knife, homogenizer, porcelain dish, Whatman No.1 filter paper, C_{18} Sep-Pak cartridge, conical flask (100mL, 250mL), 0.45µm Nylon filter, syringe filter, micropipette, HPLC system containing RI detector (Hitachi), amine polar bonded phase column, 10µm (250mm × 4.6mm I.D.).

Glucose/ Fructose/ Sucrose Stock Solution

2.5g of glucose, fructose and sucrose were accurately weighed. Each of them was dissolved in 25mL volumetric flask respectively with acetonitrile and distilled water (50:50) to the mark. Each of volumetric flasks was shaken vertically until all glucose, fructose and sucrose were completely dissolved to produce 10% glucose, fructose and sucrose stock solution.

Glucose/ Fructose/ Sucrose Standard Solution

The glucose, fructose and sucrose standard solutions, of 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 %, were prepared in 10mL volumetric flask respectively, by diluting 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 mL of glucose, fructose, and sucrose stock solution with acetonitrile and distilled water (50:50) to the mark respectively. Each of volumetric flasks was shaken vertically until the solutions were homogenized. The standard solutions of mixture of fructose, glucose and sucrose, at 0.167, 0.5, 0.833, 1.167, 1.5 and 1.833 % were prepared respectively, by pipetting 2mL of each homogenized solution from 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 % standard solutions of glucose, fructose and sucrose respectively into a vial.

Preparation of Mobile Phase

156.01mg of sodium phosphate was accurately weighed and dissolved in 100mL volumetric flask with distilled water to the mark to produce 10mM sodium phosphate (monobasic) solution. 78mL acetonitrile and 22mL 10mM sodium phosphate (monobasic) solution were mixed in a 250mL beaker. Degassing of solution was done by using ultrasonic bath.

Preparation of Sample from Fresh Fruit Juices

All fruits except for strawberries were peeled, and cut into small pieces. The fruits were then blended for 3-5min at maximum speed with a homogenizer. The homogenate was vacuum-filtered through Whatman No.1 filter paper. Then, 12.5mL filtrate was diluted with acetonitrile and distilled water (50:50) in a 50mL volumetric flask to the mark. The diluted fresh fruit juice was filtered through C_{18} Sep-Pak cartridge. Total sugars of fresh fruit juices were contained in the eluate. The eluate was filtered through 0.45 µm nylon filter before HPLC analysis.

Preparation of Sample from Packed Fruit Juices

12.5 mL of packed fruit juice was diluted with acetonitrile and distilled water (50:50) in a 50mL

volumetric flask to the mark. The diluted packed fruit juice was filtered through C_{18} Sep-Pak cartridge. Total sugars of packed fruit juices were contained in the eluate. The eluate was filtered through 0.45µm nylon filter before HPLC analysis.

Result and Discussion

Table 1. Results of Percentage of Purity and Water Content on Fructose, Glucose, and Sucrose

Tests	Fructose	Glucose	Sucrose
Purity (%)	98.69	90.01	102.5
Water Content (%)	0.8862	9.2675	0.1357

Purity tests of fructose, glucose and sucrose were conducted to know the percentage purity of the standards. Purity test was done by comparing the peak area response of sample and analytical standard solutions. Water determination test was conducted for fructose, glucose and sucrose using Karl Fischer Method. Based on Table 1, purity of fructose, glucose and sucrose were 98.69, 90.01, and 102.5% respectively whereas water content of fructose, glucose and sucrose were 0.8862, 9.2675, and 0.1357%, respectively.

System suitability tests were applied to analytical instrument. They were designed to evaluate the components of the analytical system in order to show performance indicator of the system in comparison to standards required by the method. Repeatability, resolution, capacity factor, theoretical plate and tailing factor were some common parameters that used in system suitability parameters (Kazakevich and LoBrutto 2007; David 2005)

Table 2 showed results of inter-day test for fructose, glucose, and sucrose. The inter-day test of retention time and Area Under The Curve (AUC) for fructose, glucose and sucrose showed percentages of coefficient of variance of less than 2%. Hence, the inter-day test was fulfilled the requirement for repeatability.

Based on Table 3, values of resolution, capacity factor, theoretical plate and tailing factor for fructose, glucose and sucrose were fulfilled the requirement for each parameter.

Method validation of process was carried out to confirm that the method was suited for its intended use. Linearity, limit of detection, limit of quantification, precision, accuracy, and recovery, were the parameters involved in method validation process. (David *et al.* 2005; Ahuja and Dong 2005).

The calibration curve of fructose, glucose and sucrose was constructed by plotting peak area versus concentration. The peak area was measured as average peak area which was obtained from three replicates of each concentration. In Table 4, the percentage of coefficient of variance for each concentration of fructose, glucose and sucrose was less than 2%.

Table 2. Results of Inter-day Test for Fructose, Glucose, and Sucrose

Sample	Fructose		Glucose		Sucrose	
	t_R (min)	AUC	t_R (min)	AUC	t_R (min)	AUC
1	8.733	3762052	10.013	3639734	14.600	3687240
2	8.773	3755846	9.993	3654855	14.587	3595730
3	8.727	3652159	9.987	3580006	14.560	3523888
4	8.727	3718690	9.987	3596950	14.560	3620536
5	8.673	3724392	9.927	3614296	14.427	3618626
6	8.547	3719256	9.740	3567071	13.973	3547333
% CV	**0.921**	**1.051**	**1.033**	**0.945**	**1.676**	**1.618**

*t_R (Retention Time)

Table 3. Results of System Suitability Test for Fructose, Glucose and Sucrose

Parameter	Fructose	Glucose	Sucrose	Requirement
Resolution	10.064	2.017	6.196	> 1.5
Capacity Factor	1.460	1.847	3.150	1 – 20
Theoretical Plate	4081	2460	5769	> 2000
Tailing Factor	1.093	1.081	1.167	0.9 – 1.4

Table 4. AUC of Stand ard Solution for Fructose, Glucose and Sucrose

Concentration	AUC		
	Fructose	**Glucose**	**Sucrose**
0.167	546890.667	527443.000	556941.667
0.5	1751401.667	1746825.000	1742849.333
0.833	3185830.333	3100595.333	3084566.667
1.167	4379239.000	4311246.667	4385018.000
1.5	5665955.333	5607440.000	5545969.000
1.833	6901607.667	6793668.000	6810993.667

Table 5. Results of Inter-day and Intra-day for Fructose

Day	t_R (min)	CV (%)	AUC	CV (%)	CV (t_R) Intra-day (%)	CV (AUC) Intra-day (%)
1	8.733	0.921	3762052	1.051	1.250	1.104
	8.773		3755846			
	8.727		3652159			
	8.727		3718690			
	8.673		3724392			
	8.547		3719256			
2	8.880	0.910	3740475	1.024		
	8.767		3708196			
	8.720		3707436			
	8.693		3741330			
	8.673		3696471			
	8.673		3637714			
3	8.780	1.710	3825449	1.301		
	8.767		3686158			
	8.707		3715942			
	8.667		3726348			
	8.480		3723996			
	8.433		3707677			

Table 6. Results of Inter-day and Intra-day for Glucose

Day	t_R (min)	CV (%)	AUC	CV (%)	CV (t_R) Intra-day (%)	CV (AUC) Intra-day (%)
1	10.013	1.033	3639734	0.945	1.329	1.845
	9.993		3654855			
	9.987		3580006			
	9.987		3596950			
	9.927		3614296			
	9.740		3567071			
2	10.133	0.821	3650452	0.844		
	10.027		3590183			
	9.980		3642876			
	9.947		3655404			
	9.920		3637500			
	9.920		3586810			
3	9.993	1.679	3771510	0.635		
	10.013		3639734			
	9.993		3654855			
	9.987		3580006			
	9.987		3596950			
	9.927		3614296			

Linearity was a visual representation of the relationship between detector response and the analyte concentration. (Yuri and Rosario 2007) The sensitivity was directly derived from a response versus concentration plot. A very sensitive method had a large slope, so that a small change in concentration results in a large shift in the response. Meanwhile, a low sensitivity showed a small change in response for a similar change of concentration.

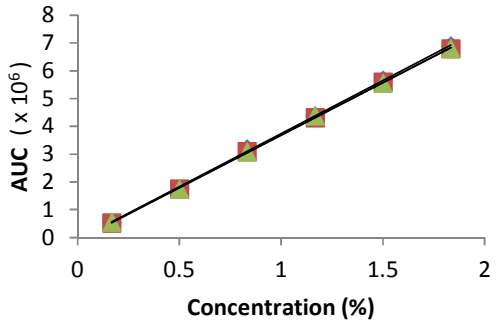

Figure 1. Calibration Curves of Fructose, Glucose and Sucrose.

From Figure 1 showed that the obtained regression equations for fructose, glucose and sucrose were y = 3833208.4806x – 94721.0361, y = 3782886.4708x – 101683.4708, y = 3770593.9638x – 82870.9083 with regression coefficients, r^2 of 0.9995, 0.9997 and 0.9996 respectively. The calculated regression function coefficients, Vx0 for fructose, glucose and sucrose were 0.0152, 0.0112 and 0.0132 %. The requirement for linearity is r^2~1 and Vx0 less than 2%. Hence, the requirement for linearity of fructose, glucose and sucrose were fulfilled.

Limit of detection (LOD) was the smallest amount of an analyte which can be detected by a particular method. Limit of quantification (LOQ) was the smallest amount of analyte which can be quantified reliably. A good LOD and LOQ had a value which was smaller than the lowest value in concentration range in calibration curve. (David 2005) The calculated LOD and LOQ from calibration curve for fructose, glucose and sucrose were 0.0457 and 0.1523, 0.0336 and 0.112, 0.0397 and 0.1324, respectively. Thus, the LOD and LOQ for fructose, glucose and sucrose had a value smaller than the lowest value in concentration range in calibration curve which was 0.167%.

Precision expressed the closeness of the agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision was determined by calculating the percentage coefficient of variance for inter-day and intra-day. In general, the percentage coefficient of variance of less than 2% was desirable. (Ahuja and Dong 2005) Based on Table 5, Table 6 and Table 7, the percentage of coefficient of variances for inter-day and intra-day of retention time and AUC show less than 2%. Thus, the requirement of precision was met.

Table 7. Results of Inter-day and Intra-day for Sucrose

Day	t_R (min)	CV (%)	AUC	CV (%)	CV (t_R) Intra-day (%)	CV (AUC) Intra-day (%)
1	14.600	1.676	3687240	1.618	1.868	1.461
	14.587		3595730			
	14.560		3523888			
	14.560		3620536			
	14.427		3618626			
	13.973		3547333			
2	14.747	0.821	3578225	0.637		
	14.640		3615586			
	14.560		3630350			
	14.493		3643338			
	14.440		3598837			
	14.453		3609794			
3	14.440	1.972	3715819	0.707		
	14.413		3688972			
	14.360		3695772			
	14.133		3678408			
	13.880		3637320			
	13.800		3686271			

Table 8. Results for Accuracy and Precision in Apple Juice

Components	C (%)	Average AUC	C'	Recovery (%)	Average Recovery (%)	CV (%)
Fructose	0	2677542.000	0.723		88.572	1.896
	0.5	4338870.333	1.157	86.681		
	1	6094727.667	1.615	89.147		
	1.5	7845935.000	2.072	89.888		
Glucose	0	1864804.667	0.520		88.836	1.906
	0.5	3508422.667	0.954	86.898		
	1	5253554.333	1.416	89.581		
	1.5	6973367.000	1.870	90.029		
Sucrose	0	5027264.333	1.355		96.153	1.742
	0.5	6809644.333	1.828	94.541		
	1	8648328.333	2.316	96.034		
	1.5	10563505.000	2.824	97.885		

Table 9. Results of Fructose, Glucose, and Sucrose Contents in Packed Fruits

Packed Juices	Concentration (%)		
	Fructose	Glucose	Sucrose
Orange	0.469	0.454	1.861
Apple	0.714	0.468	1.389
Kiwi & Grape	1.303	1.233	0.889
Strawberry	1.222	1.169	0.556
Guava	1.431	1.286	0.544

Table 10. Results of Fructose, Glucose, and Sucrose Contents in Fresh Juices

Packed Juices	Concentration (%)		
	Fructose	Glucose	Sucrose
Orange	0.309	0.261	0.898
Apple	1.587	0.745	0.334
Kiwi & Grape	0.826	0.762	0.324
Strawberry	0.466	0.357	0.063
Guava	0.579	0.285	0.505

Accuracy was an agreement between the result obtained with method being validated and an accepted reference value. Accuracy was reported as percentage recovery in relation to known amount of analyte added to the sample or as the difference between the known amount and the amount determined by analysis (Yuri and Rosario 2007; Ahuja and Dong 2005). Therefore, the percentage recovery of fructose, glucose and sucrose added into the apple juice was calculated. The precision of sample was determined by calculating the percentage coefficient of variance.The percentage of coefficient of variance of less than 2% was desirable.

Based on Table 8, the percentage recovery of fructose, glucose, and sucrose using standard addition method was found to be in a range of 86.681 – 89.888, 86.898 – 90.029, and 94.541 – 97.885 %. The percentage of coefficient of variances for fructose, glucose, and sucrose were 1.896, 1.906, and 1.742%. The percentage of coefficient of variances were less than 2%. Thus, the precision of sample was met.

Results of fructose, glucose and sucrose in packed juices were shown in Table 9. Guava juice contained the highest percentage of fructose and glucose, but contained the lowest percentage of sucrose. Orange juice contained the highest percentage of sucrose, but contained the lowest percentage of fructose and sucrose.

Table 10 showed the results of fructose, glucose, and sucrose contents in fresh juices. Orange juice contained the lowest percentage of fructose and glucose, but contained the highest percentage of sucrose. While apple juice and kiwi juice contained the highest percentage of fructose and glucose

respectively. Strawberry juice contained the lowest percentage of sucrose.

Conclusion

High performance liquid chromatography was suitable to determine sugar content in fruit juices. The optimum conditions for the analysis were as follow: a NH_2 polar bonded phase column, 10 µm (250 mm × 4.6 mm I.D.) and the column temperature was 43.5 °C, a mobile phase composed of acetonitrile and 10mM sodium phosphate (monobasic) solution (78:22), a flow rate of1.0mL/min, a Refractive Index detector, and injection volume was 20 µL. The method validation showed good linearity of equations for fructose, glucose and sucrose were y=3833208.4806x–94721.0361, y=3782886.4708x–101683.4708, y=3770593.9638x–82870.9083 with regression coefficients, r^2 of 0.9995, 0.9997 and 0.9996 respectively. The calculated regression function coefficients, Vx0 for fructose, glucose and sucrose were 0.0152, 0.0112 and 0.0132 %. The percentage recovery of fructose, glucose, and sucrose was found to be in a range of 86.681 – 89.888, 86.898 – 90.029, and 94.541 – 97.885 %. Fructose, glucose and sucrose of fresh juices contained 0.469 – 1.431, 0.454 – 1.286, 0.544 – 1.861 %, whereas that of packed juices contained 0.309 – 1.587, 0.261 – 0.762, 0.063 – 0.898 %, respectively.

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