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Determination of Sugar Profiles of Sweetened Foods and Beverages

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Abstract The determination of sugar profile in commonly consumed sweetened foods and beverages (cake, chocolate, jelly tots, cookie, wafer, pudding, fruit yogurt, limon-flavored soda, cola, lemonade, mineral water, fruit juice, milk drink and ice tea) was carried out using high performance liquid chromatography (HPLC). The amount of fructose was found higher than the amount of glucose in most of the foods and beverages (juice, limon-flavored soda, mineral water, cola, chocolate, cookie, wafer). The highest fructose contents were found in cola (71.10 % of sugars), chocolate (52.40 % of sugars) and limon-flavored soda (48.21 % of sugars) samples. The galactose, mannitol, arabinose and xylitol were not detected in any of the examined food and beverage samples. The predominant sugar in milk drinks, jelly tots, pudding, cookie and cake samples was determined as sucrose. Maltitol was only determined in cake and jellytots samples.

Keywords: *fructose, sweetened foods, sweetened beverages, sugar profile, HPLC*

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1. Introduction

Dietary fructose is a naturally occurring sugar in fruits and vegetables. However, fructose is commonly added to foods and drinks for palatability and taste enhancement, and for browning of some foods, such as baked goods. The primary reason that fructose is used commercially in foods and beverages, besides its low cost, is its high relative sweetness. It is the sweetest of all naturally occurring carbohydrates. It does not make up satiety and fullness [9].

The consumption of sugar has dramatically increased in the worldwide, according to data from the U.S. in 2008, people are consuming over 60 pounds (28 kg) of added sugar per year and this does not include fruit juices. An increase in fructose consumption over the past 10 to 20 years has been linked to a rise in metabolic disorders such as cardiovascular diseases [8], type 2 diabetes [3], hypertension [5], dyslipidemia [10], gout [2] and obesity [7]. In order to reduce the frequency of metabolic disorders in all ages, the amount of fructose in processed foods and beverages should also be taken into consideration. Fructose can only be metabolized by the liver and can't be used for energy by body's cells. It's therefore not only completely useless for the body, but is also a toxin in high enough amount because the job of the liver is to get rid of it, mainly by transforming it into fat and sending that fat to our fat cells [4].

There are currently no disclosures of fructose content in foods and beverages, and many nutrition databases only rely on product label information, it is challenging to accurately determine actual fructose consumption levels in nutrition research.

The objective of this study was to determine fructose content and sugar profile in commonly consumed sweetened foods and beverages (cake, chocolate, jelly tots, cookie, wafer, pudding, fruit yogurt, limon-flavored soda, cola, lemonade, mineral water, fruit juice, milk drink and ice tea).

2. Materials and Methods

2.1. Reagents and Standards

Fructose, glucose, sucrose, lactose, maltitol, sucralose, mannitol, xylitol, galactose, arabinose were obtained from Merck (Darmstadt, Germany). All standards were more than 99 % pure. A standard solution of 10 g L^{-1} of each compoundwas prepared in aqua. Calibration standards were prepared in the range of 1.0 – 10 and 0.01 – 0.5 g L^{-1}. In all cases, the correlation coefficients of linear functions were > 0.9950. The calibration curves were created from six calibration standards.

2.2. Sample Collection

All of the food and beverage samples (cake, chocolate, jelly tots, cookie, wafer, pudding, fruit yogurt, limon-flavored soda, cola, lemonade, mineral water, fruit juice, milk drink and ice tea) which are 5 different brands were taken from different local markets in Turkey. All analyses were repeated three times for each sample.

2.3. Sample Preparation

Liquid samples: After the beverage had been degassed in an ultrasonic bath, an aliquot of either 2 mL was diluted 1:4 with ultrapure water. The mixtures were centrifuged at

1400 rpm for 15 min. Cleanup procedure for samples containing proteins was applied. The supernatant after passing through a membrane filter (0.45 µm, PVDF, Millex, Ireland) was injected into HPLC system [11].

Powdered samples: Five gram of the homogenized sample was mixed with 10 mL of ultrapure water in a 25 mL polypropylene centrifuge tube. After 5 min shaking, the supernatant liquid was removed with a Pasteur pipet and the solid residue was extracted for a second time with 10 mL of ultrapure water. The combined supernatants after passing through a membrane filter (0.45 µm, PVDF, Millex, Ireland) was injected into HPLC system. Cleanup procedure for samples containing proteins before passing through a membrane filter was applied. [1].

Cleanup procedure: 2mL Carrez solution no. 1 (15 g of K4[Fe(CN)6].3H2O in water diluted at 100 mL) was added over the supernatant. After shaking, 2 mL Carez no. 2solution (30 g zinc sulfate (ZnSO4.7H2O) are diluted in water at 100 mL) was added. Thesolution was shaken and it was kept at the room temperature for 10 minutes. The clarifiedsample mixture was centrifuged for 10 minutes. The settled matter was washed twice with water and it was centrifuged again, each of the supernatants was collected in the 50 mL volumetric flask and the solution was then diluted to the mark with water [6].

2.4. HPLC Analysis

HPLC analysis was performed on a Shimadzu C196-E039A instrument, equipped with a CARBOSepCoregel 87P column 8 µm (7.8 x 300 mm) (Transgenomic, USA). The refractive index dedector (RID-10A) was used. Data processing was carried out with LCsolution software. The mobile phase was DI H_2O with a flow rate of 1 mL min^{-1}. The column temperature was 85°C [11].

2.5. Quality Control and Quality Assurance

The calibration curves were plotted by the peak area versus concentration of each sugar. Limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined on the basis of response and slope of each regression equation at a signal-to-noise ratio of 3 and 10, respectively. The recovery test was used to evaluate the accuracy of applied method. Selected samples were spiked with known amounts of fructose, glucose, sucrose, lactose, maltitol, mannitol, xylitol (each for 100 mg L^{-1}), and then analysed as described above. The average recoveries were calculated by the following equation:

$$\text{Recovery}(\%) = \frac{\begin{pmatrix}\text{observed amount}\\ -\text{original amount}\end{pmatrix}}{\text{spiked amount}} \times 100$$

Linearity, LOD, LOQ, recovery and relative standard deviations (RSD) are listed in Table 1.

2.6. Statistical Evaluation

All statistical analyses were performed using the SPSS for Windows, version 22.0 (Chicago, IL, USA) and probability value of less than 0.05 was accepted as statistically significant. Data were expressed as mean ± standard deviation (SD). Data were analyzed using analysis of variance (ANOVA) followed by Bonferroni's post hoc test. When the null hypothesis could be rejected, comparisons between the two groups were made with the Mann–Whitney *U* non-parametric test for independent samples.

Table 1.Retention time,linearity, LOD, LOQ, recovery and relative standard deviations (RSD) values of examined sugars

Sample	Retention time (min)	Linearity (R^2)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Recovery (%)	RSD (%)
Sucrose	11.76	0.9956	0.280	0.924	97-101	6-10
Lactose	12.93	0.9987	0.264	0.871	94-100	6-10
Glucose	14.08	0.9997	0.229	0.756	99-102	6-10
Galactose	16.66	0.9956	0.269	0.888	98-103	6-10
Arabinose	17.75	0.9996	0.232	0.766	94-100	6-10
Fructose	19.63	0.9983	0.286	0.944	93-101	6-10
Maltitol	22.67	0.9998	0.216	0.713	94-101	6-10
Sucralose	24.24	0.9992	0.233	0.769	98-103	6-10
Mannitol	30.52	0.9997	0.219	0.723	94-100	6-10
Xylitol	39.88	0.9997	0.230	0.759	99-102	6-10

3. Results and Discussion

Under the applied HPLC conditions, the retention times of sucrose, lactose, glucose, galactose, arabinose, fructose, maltitol, sucralose, mannitol and xylitol were 11.76, 12.93, 14.08, 16.66, 17.75, 19.63, 22.67, 24.24, 30.52 and 39.88 min, respectively. The chromatogram of standard substances is given in Figure 1.

Sugar concentrations of the examined beverage samples are presented in Table 2. The contents of fructose in limon-flavored soda (6.19 g /100 mL), ice tea (7.73 g / 100 mL), and cola (7.65 g / 100 mL) samples were found very high. The predominant sugar in lemonade (11.94 g / 100 mL), milk drink (10.38 g / 100 mL) and juice (9.43 g / 100 mL) samples was determined as sucrose. The mannitol, maltitol, galactose, arabinose and xylitol were not detected in the examined beverage samples. The sucralose and lactose were detected in small amounts in these products. The chromatogram belonging to one of the examined beverage samples (peach juice) is given in Figure 2.a.

Sugar contents of the examined food samples are listed in Table 3. Very high levels of fructose (24.68 g/100 g) were found in chocolate samples. The predominant sugar in all of the examined food samples was determined as sucrose. The mannitol, galactose, arabinose and xylitol were not detected in any of the examined food samples. The sucralose was detected in only cake samples. Maltitol was also detected in cake and jelly tots samples. The chromatogram belonging to one of the examined food samples (wafer) is given in Figure 2.b.

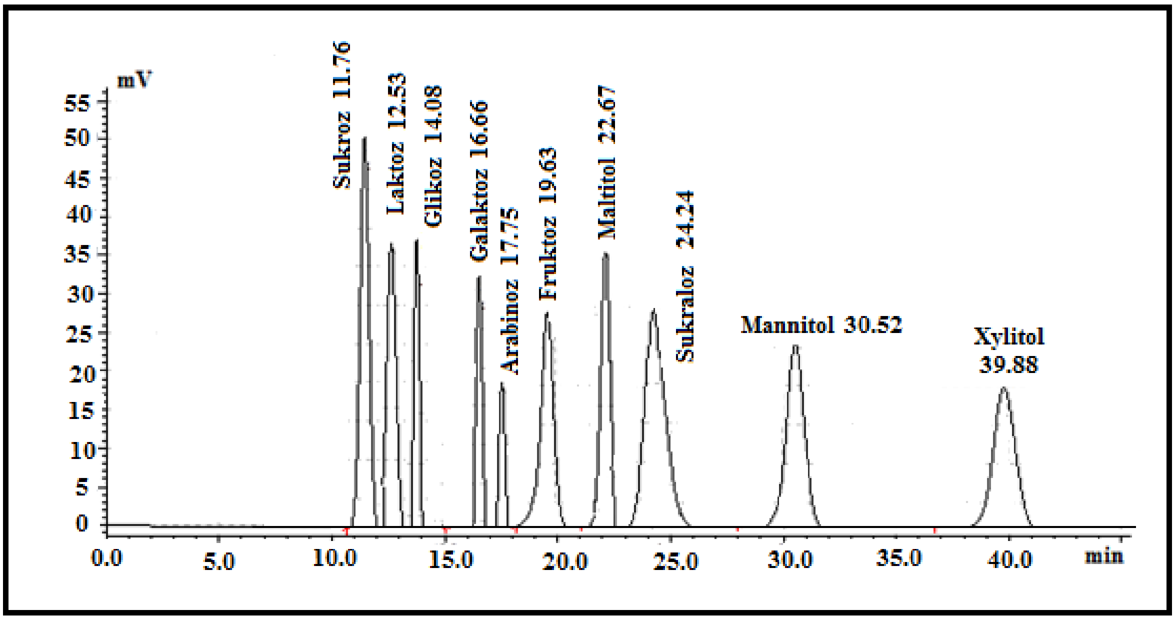

Figure 1. The chromatogram of standard substances

Table 2. Sugar concentrations of the examined beverage samples (g/100 ml)

Beverage		Fructose	Glucose	Sucrose	Lactose	Sucralose	Total Sugar
Peach Juice	Max	6.72	4.23	21.12	nd*	nd	24.21
	Min	1.07	0.96	3.69	nd	nd	12.19
	Mean	2.69	2.05	10.91	nd	nd	15.65
Cherry Juice	Max	11.18	12.00	nd	nd	0.21	23.20
	Min	3.28	2.52	nd	nd	0.02	5.89
	Mean	6.73	5.85	nd	nd	0.13	12.71
Apricot Juice	Max	4.22	2.51	18.40	nd	nd	21.30
	Min	0.80	0.83	7.16	nd	nd	10.20
	Mean	1.74	1.43	11.67	nd	nd	14.84
Mixed Fruit Juice	Max	12.06	2.35	6.70	nd	0.01	13.01
	Min	2.49	1.48	4.22	nd	0.01	8.20
	Mean	5.11	1.60	4.57	nd	0.01	11.29
Lemonade	Max	1.35	1.20	12.43	nd	nd	14.79
	Min	1.10	0.98	11.49	nd	nd	13.72
	Mean	1.21	1.14	11.94	nd	nd	14.29
Soda	Max	11.06	16.17	2.09	nd	0.01	16.94
	Min	5.63	3.67	0.60	nd	nd	11.02
	Mean	6.19	5.95	0.69	nd	0.01	12.84
Mineral Water	Max	7.10	5.06	11.60	nd	0.34	13.26
	Min	1.66	0.52	8.35	nd	nd	4.92
	Mean	3.34	1.48	3.99	nd	0.07	8.88
Cola	Max	13.70	4.60	5.37	nd	nd	13.70
	Min	0.92	3.43	2.12	nd	nd	6.29
	Mean	7.65	1.61	1.50	nd	nd	10.76
Chocolate Milk	Max	nd	nd	11.05	1.11	0.04	12.20
	Min	nd	nd	8.55	0.86	0.03	9.44
	Mean	nd	nd	9.69	1.02	0.04	10.75
Banana Milk	Max	nd	nd	11.01	1.86	nd	12.87
	Min	nd	nd	10.15	1.71	nd	11.86
	Mean	nd	nd	10.57	1.78	nd	12.35
Strawberry Milk	Max	nd	nd	12.14	1.22	0.04	13.40
	Min	nd	nd	9.69	0.97	0.03	10.69
	Mean	nd	nd	10.89	1.08	0.04	12.01
Ice tea	Max	8.14	1.10	2.30	nd	0.03	11.45
	Min	7.36	0.76	2.05	nd	0.01	10.34
	Mean	7.73	0.87	2.17	nd	0.02	10.79

*nd = not detected

Table 3. Sugar contents of the examined food samples (g/100 g)

Food		Fructose	Glucose	Sucrose	Lactose	Sucralose	Maltitol	TotalSugar
Jelly tots	Max	6.00	38.80	44.77	6.42	nd*	0.06	93.47
	Min	3.79	4.33	33.83	5.17	nd	0.03	48.19
	Mean	4.99	11.55	38.57	5.75	nd	0.03	60.89
Chocolate	Max	27.82	nd	15.86	12.86	nd	nd	53.10
	Min	21.95	nd	9.03	6.51	nd	nd	41.90
	Mean	24.68	nd	12.45	9.97	nd	nd	47.10
Pudding	Max	0.71	1.92	23.67	nd	nd	nd	26.30
	Min	0.45	1.22	15.03	nd	nd	nd	16.70
	Mean	0.54	1.46	17.98	nd	nd	nd	19.98
Yogurt	Max	2.05	1.30	11.48	0.90	nd	nd	15.65
	Min	1.03	1.16	10.58	0.78	nd	nd	12.89
	Mean	1.18	1.22	10.93	0.85	nd	nd	14.18
Cookie	Max	22.30	11.98	34.55	nd	nd	nd	41.20
	Min	0.07	1.12	16.00	nd	nd	nd	17.98
	Mean	6.82	2.62	17.75	nd	nd	nd	27.19
Wafer	Max	31.50	11.20	22.20	14.70	nd	nd	46.20
	Min	7.10	6.60	19.80	2.40	nd	nd	40.00
	Mean	12.52	8.04	16.92	5.50	nd	nd	42.98
Cake	Max	0.02	4.20	65.60	2.10	24.00	4.00	75.60
	Min	nd	0.05	21.00	1.33	0.80	0.50	45.50
	Mean	0.02	2.40	50.85	0.69	4.96	1.50	60.42

*nd= not detected.

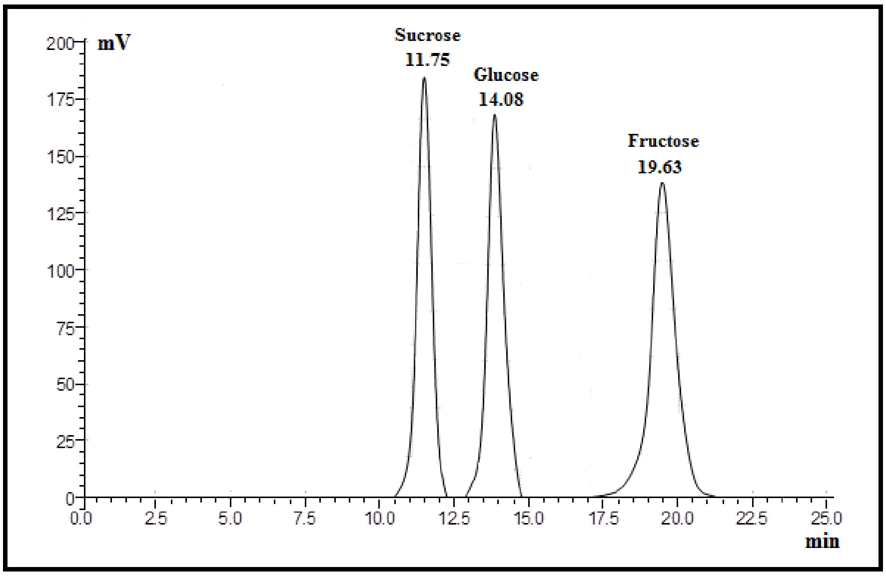

Figure 2.a. The chromatogram of peach juice

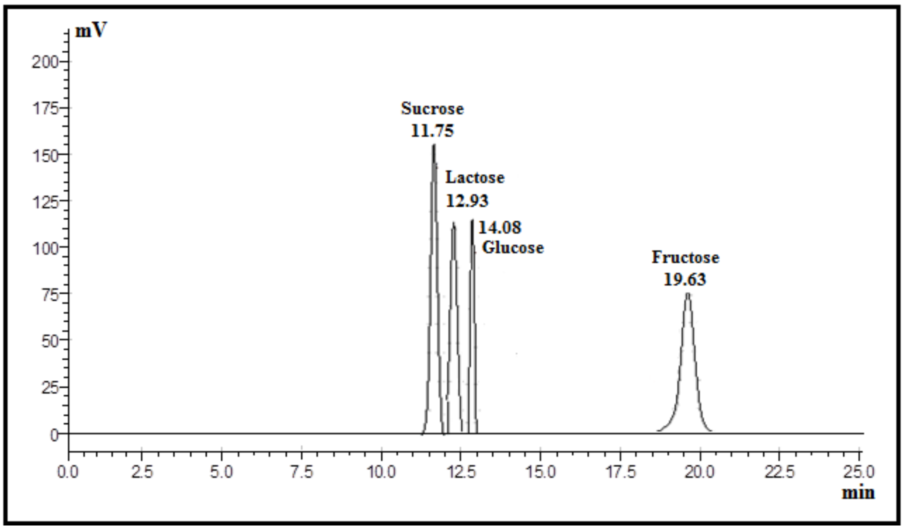

Figure 2.b. The chromatogram of wafer

Table 4 is summarized the sugar profiles of the examined food and beverage samples. The results obtained have shown that the sucrose was the major component of total sugar in some foods and beverages such as jelly tots, pudding, cookie, cake, milk drinks. The sucralose and maltitol levels in all of the examined samples were very little.

Table 4. Sugar profiles of the examined food and beverage samples (presented in %)

Sample	Fructose	Glucose	Sucrose	Lactose	Sucralose	Maltitol
Juice	38.47	24.43	37.01	0.00	0.09	0.00
Soda	48.21	46.34	5.37	0.00	0.08	0.00
Mineral water	37.61	16.67	44.93	0.00	0.79	0.00
Cola	71.10	15.00	13.90	0.00	0.00	0.00
Milk	0.00	0.00	88.82	10.83	0.35	0.00
Jelly tots	8.19	18.97	63.34	9.45	0.00	0.05
Pudding	2.70	7.30	90.00	0.00	0.00	0.00
Chocolate	52.40	0.00	26.43	21.17	0.00	0.00
Cookie	25.08	9.63	65.29	0.00	0.00	0.00
Wafer	29.13	18.71	39.36	12.80	0.00	0.00
Cake	0.03	3.97	84.17	1.14	8.21	2.48

Cola samples consistently contained 71.10 % of total sugar as fructose despite neither high fructose corn syrup (HFCS) nor fructose being listed on the label. Additionally, chocolate, limon-flavored soda, juice, mineral water, wafer, all which list neither HFCS nor fructose as added sweeteners, contained fructose as a percentage of total sugar in 52.40%, 48.21 %, 38.47 %, 37.61 %, and 29.13 %, respectively.

The mean fructose and glucose levels in cherry juice were significantly higher compared with peach juice and apricot juice (6.73 vs 2.69 $p < 0.05$; 6.73 vs 1.74 $p = 0.016$; 5.85 vs 2.05 $p = 0.028$; 5.85 vs 1.43 $p < 0.01$ respectively). Statistical differences between fructose and sucroselevels were observed in chocolate and pudding (24.68 vs 0.54 $p < 0.01$; 12.45 vs 17.98 $p = 0.016$). The mean fructose, sucrose and lactose levels in chocolate were significantly higher than those in cake and jelly tots (24.68 vs 0.02 $p < 0.01$; 24.68 vs 4.99 $p < 0.01$; 12.45 vs 50.85 $p < 0.01$; 12.45 vs 38.57 $p < 0.01$; 9.97 vs 0.69 $p < 0.01$; 9.97 vs 5.75 $p < 0.01$). Statistical differences between fructose, sucrose and lactose levels were observed in wafer and cake (12.52 vs 0.02 $p < 0.01$; 16.92 vs 50.85 $p = 0.028$; 5.50 vs 0.69 $p < 0.01$). The mean fructose level in chocolate was significantly higher compared with cookie (24.68 vs 6.82 $p = 0.016$). Statistical differences between fructose and sucrose levels were observed in cookie and cake (6.82 vs 0.02 $p < 0.01$; 17.75 vs 50.85 $p = 0.016$).

In the literature, not many studies had focused on the fructose content in foods and beverages. The study nearest to our study was displayed by Walker et al. (2014). They reported fructose content of 0.728 – 7.231 g / 100 mL; glucose content of 0.624 – 5.951 g / 100 mL; maltose content of 0.00 – 0.209 g / 100 mLand sucrose content of 0.00 – 8.736 g / 100 mL in carbonated beverages. These values were lower than our study. Ventura et al. (2011) found 6.3 g/100 mL fructose, 4.43 g/100 mL glucose, <0.05 g/100 mL sucrose in cola samples, 6.4 g / 100 mL fructose, 4.13 g / 100 mL glucose, < 0.05 g /100 mL sucrose in limon-flavored soda samples and 4.94 g / 100 mL fructose, 4.23 g / 100 mL glucose, 0.53 g /100 mL sucrose in fruit juice samples. In our study, 7.65 g/100 mL fructose, 1.61 g/100 mL glucose, 1.50 g/100 mL sucrose were found in cola samples. 6.19 g/100 mL fructose, 5.95 g/100 mL glucose, 0.69 g/100 mL sucrose were found in limon-flavored soda samples. In the literature, no previous research related to sugar profiles of foods. The biggest advantage of this study was being the first study in the literature for the determination of sugar profiles of foods.

Results showed that the total sugar content of the all samples ranged from 97 to 110 % which was listed on the food label. Actual fructose levels are difficult to estimate because of the unlabeled quantity of fructose in foods and beverages. Such studies are very important because of determining the types of sugar actually used in foods and beverages. This study will be an example for studies related to determination of sugar profiles of foods and beverages consumed in different countries.

Conflict of Interest

Sana Sungur declares that she has no conflict of interest. Yusuf Kılboz declares that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

Informed Consent

Not applicable.

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