Determination of Sugars and Alcohols in Apple Juice and Cider by High Performance Liquid Chromatography

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Key Words

Column Liquid Chromatography

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Summary

The application of a high-performance liquid chromatographic procedure to the separation and determination of major sugars, sorbitol, glycerol and ethanol in apples, apple juice and cider is described. The HPLC system consisted of a cation-exchange resin column in calcium form, a solvent system of an aqueous solution with calcium EDTA and a refractive index detector. The analysis was completed after 16 minutes. A recovery greater than 91 % was observed for all compounds with most recoveries nearing 100 %. The coefficients of variation ranged from 2 % to 6 %.

Introduction

Carbohydrates are among the most abundant compounds found in nature, and the analysis of sugars and sugar mixtures is of considerable importance to the food industry. The analysis of major sugars in apple, apple juice and cider is of great interest for several reasons: firstly, the sugars, together with the sorbitol, can be used as indicators of apple maturity because the relative ratio between the different major sugars changes in the course of the ripening, e.g., fructose, the principal sugar of reserve, is synthesized from sorbitol during ripening by the action of sorbitol dehydrogenase and glucose is accumulated at the end of maturity due to starch hydrolysis. On the other hand, during the production of ciders it is necessary to control the free sugars together with the ethanol and glycerol concentrations because all these factors contribute to the flavour and to the sensory characteristics of the ciders in general. Many ciders are obtained by mixing several

varieties of apples with different contents of sugars and other components. Finally, the sugars are important for the nutritive value and they can be used for the detection of adulteration, carried out by adding corn, cane or beet sugar to apple juice. This type of adulteration can be easily detected because a true apple juice should contain a minimum fructose/glucose ratio of 1.6 and a maximum sucrose content of 3.5 %.

Many different analytical techniques have been investigated for the separation and determination of sugars. Of the different techniques available, enzymatic and chromatographic procedures are most commonly used. The latter include paper chromatography, thin-layer chromatography, gas-liquid chromatography, and more recently, high performance liquid chromatography. Lee [1] has reviewed the many methods available for determining carbohydrates in foods. A large number of these methods are time-consuming and difficult to quantitate or require some derivatization before analysis. In recent years the application of HPLC to many fields of analytical chemistry has allowed the rapid characterization of many nutritionally significant carbohydrates in food products.

Several authors have studied and described the mechanisms of sugar separation by HPLC with various stationary phases: bare silica [2, 3], bonded aminopropyl silica [4, 5, 6], cation-exchange resin [7, 8], anion-exchange resin [9] and copper silicate gel [10]. Each stationary phase has particular advantages, as have the various detection methods: differential refractometry, UV absorptiometry at low wavelength and post-column derivatization. Rosset et al [11] have published a critical review of the several mechanism and detection systems used in sugar separation by high performance liquid chromatography concluding that the most efficient method is based upon the use of cation exchange resins in spite of their high cost.

This paper is specifically concerned with the application of HPLC methods to the control and the determination of polyalcohols (glycerol and sorbitol), ethanol and major sugars found in apples and some industrial derivatives. It describes a rapid procedure for extracting and quantifying glucose, fructose, sucrose and sorbitol in apples using a cation-exchange resin column (Ca^{2+}) and refractive index

detection. The same compounds are determined in apple juice in three stages of the fermentation process, although the course of alcoholic fermentation can be followed by monitoring the reduction of fermentable sugars and the production of alcohol and glycerol.

The HPLC method has been evaluated by triplication of data and by spiking samples and also, for the glucose and fructose, by a comparison of results with data obtained by enzymatic analysis.

Experimental

The chromatographic system consisted of a Water 510 pump; a Wisp Model 712 automatic injector; a Water 410 RI detector; and a Digital 380 data station.

The columns were a μ -Bondapak Carbohydrate column, 300 mm X 4 mm i.d. (Water Associates), a Trazer Sugars column, 300 mm X 7 mm i.d. (Tecnokroma), and a Sugar Pak I column, 300 mm X 6.5 mm i.d. (Water Associates). Column temperature was controlled with a Selecta Digiterm 542 circulating water bath.

The mobile phases were acetonitrile/water (83 : 17) for the Carbohydrate column and an aqueous solution containing 50 ppm of calcium salt of EDTA for the Trazer and Sugar Pak I columns, each degassed with helium prior to use.

Standard solutions of carbohydrates (D (+) sucrose, D (-) fructose and D (+) glucose), polyalcohols (D-sorbitol and glycerol) and ethanol were prepared by dissolving their analytical grade reagents (purchased from Sigma Chemical Co. St. Louis, MO 63178).

Preparation of Samples

Apple extracts were prepared as described by Richmond et al. [12] with some modifications in order to ensure the extraction of other components present such as phenolic compounds, for further studies. 1 Kg of apple samples were selected. One quarter of each apple was excised and placed in a blender. The fruit was covered with sufficient ethanol and HCl was added to make the final concentrations 80 % and 1 % respectively. Fruit and ethanol were blended for two minutes at high speed. The resulting slurry was refluxed with stirring and under an inert atmosphere for 2 h. The extract was filtered through a gooch crucible (no. 3) and was washed by adding 80 % ethanol. It was then reduced to a volume of approximately 250 ml in a rotary vacuum evaporator. After that, the fruit concentrate was made to 500 ml with 80 % ethanol.

Apple extracts and apple juice were filtered through a Sep-Pak C18 cartridge (Water Associates), pretreated with methanol, to retain color pigments. Before the injection, all the samples were filtered through a 0.45 μ m Millex membrane.

Results and Discussion

Reversed-phase chromatography is one of the most important types of chromatography in carbohydrate separation. Alkylated [13] and aminoalkylated [4-6] silica gels

are among the most frequently used stationary phases in combination with water/methanol or water/acetonitrile as mobile phase. Initial separations of sugar (glucose, fructose and sucrose), ethanol and polyalcohol (sorbitol and glycerol) samples in an aminoalkylated silica gel column (Carbohydrate column), with acetonitrile/water (83 : 17) as mobile phase showed poor separations of components with a large overlapping of the glucose and the sorbitol. Moreover the fructose and the glucose were inadequately separated.

Another stationary phase used in this type of separation was an ion exchanger made of porous polymer. A cation exchanger in calcium form is commercially available for

Fig. 1
Chromatograms of standard solution of: 1 = malic acid; 2 = sucrose; 3 = glucose; 4 = fructose; 5 = glycerol; 6 = ethanol; 7 = sorbitol. Column: (A) = Trazer Sugars, 300 X 7 mm I.D. (90 °C), (B) = Sugar Pak I, 300 X 6.5 mm I.D. (90 °C). Mobile phase: water containing 50 ppm Ca (Na)₂ EDTA. Flow rate: 0.5 ml/min. Injection volume: 10 μ l.

use with samples such as those of fermentation processes and food manufacturing. In this work we used two different columns with the same packing and an aqueous solution containing 50 ppm of calcium EDTA as mobile phase. The calcium ions were added to maintain the equilibrium and prevent inversion of some sugars, because hydrogen or other cations can replace the calcium and cause inversion of sugars such as sucrose in the column. The chelating agent, EDTA, was added to the mobile phase to help overcome any potential heavy metal contamination of the column resin. Figure 1 shows the results obtained with both columns.

Comparison of the chromatograms indicates that better separation was attained with the second column (Sugar Pak I) since with the first column the malic acid and the glucose were not separated while with the Sugar Pak I column a good separation of the major sugars and the organic acids (eluted jointly at the beginning) was obtained although the ethanol and the sorbitol overlap slightly.

Conditions were optimized by varying the column temperature and the eluent flow-rate. Figure 2 shows that lowering the temperature to 80 °C results in slightly longer retention times and better resolution. In fact, ethanol and sorbitol can be separated from each other as well as from the major sugars and glycerol, a separation which is difficult to achieve with other conditions. The optimum mobile phase flow rate was found to be 0.5 ml/min. Separation of all solutes was complete after 16 minutes. When the flow rate was decreased to 0.4 ml/min resolution was not notably better and analysis time was increased by 4 minutes. Increasing the flow rate decreased total analysis time but required excessive pressure.

The optimum overall operating conditions for the HPLC system, using a Sugar Pak I column, were as follows: flow rate: 0.5 ml/min; column temperature: 80 °C; detector temperature: 37 °C; detector sensitivity: 32. System back pressure was of the order of 1.000 psi at a flow rate of 0.5 ml/min.

Quantification and Recovery

Typical chromatograms for the apple extract and apple juice in three stages of fermentation, using the operating conditions specified are shown in Figures 3 and 4. All compounds in the samples are well resolved, easily lending themselves to quantification in spite of the large amount of ethanol employed in the apple extraction procedure. Furthermore, the chromatograms enable the course of the fermentation to be followed quickly and efficiently. As can be seen the sucrose disappears in the third stage of fermentation while the concentration of the other sugars decreases markedly as the glycerol and ethanol concentration increases.

On the basis of the optimization study, the quantification of solutes was achieved by using an external standard method. Standards were injected and the integrator response factors were computed. These were then stored in the integrator for the computation of unknown concentrations. Injection volumes of 10 µl were employed for all quantita-

Fig. 2
Effect of temperature on the retention of solutes. Column, Sugar Pak I, 300 × 6.5 mm I. D. Other conditions as in Fig. 1. (A) = 80 °C; (B) = 85 °C; (C) = 90 °C.

Fig. 3

Typical chromatogram of sugars and sorbitol in apple extract. Column and chromatographic conditions as in Figure 2. Temperature column 80 °C. 1 = organic acids; 2 = sucrose; 3 = glucose; 4 = fructose; 5 = sorbitol.

tive evaluations. The detector response to varied concentrations of each solute was linear throughout the range of concentration found in the injected samples. A recalibration was performed regularly to obtain new response factors.

In order to determine the precision of the method three replicates from each type of sample were analysed and the coefficients of variation were found to be less than 6 %.

To study the accuracy of the method, recovery experiments was performed. Known amounts of sucrose, glucose, fructose, sorbitol, glycerol and ethanol were added to a variety of samples and the resulting spiked samples were subjected to the entire analytical sequence. Each solute was spiked at three different concentrations and the results for an apple juice in three progressive stages of fermentation (A, B, C) are given in Table I. The average recoveries of between 91 % and 105 % indicate that the method has an adequate degree of accuracy for the analysis of these solutes. Similar results for sugars and sorbitol were obtained for recovery studies in apple extracts.

In order to check the validity of the procedure, the enzymatic method developed by Boehringer Mannheim [14] was used for some solutes (glucose and fructose) in order to make a comparison with the HPLC procedure. The results obtained are set out in Table I, parenthetically under the data obtained by the chromatographic method. As can be seen these analyses showed good agreement.

Conclusions

HPLC is a rapid and simple technique for the determination of sugars and polyalcohols in foods and beverages. Amino-bonded silica columns are inadequate for the separation of

Fig. 4

Typical chromatograms of sugars and alcohols in apple juice in three stages of fermentation. Other conditions as in Fig. 2. Temperature column 80 °C. 1 = organic acids; 2 = sucrose; 3 = glucose; 4 = fructose; 5 = glycerol; 6 = ethanol; 7 = sorbitol.

Table I. Recovery study of sugars, sorbitol, glycerol and ethanol added to apple juice in three stages of fermentation.

Compound	Apple Juice	Amount in Juice (g/l)	Amount Added (g/l)	Found (g/l)	% Recovery $\pm$ S.D.
Sucrose	A	2.42	10.00	12.40	99.8 $\pm$ 1.9
			20.00	20.94	93.4 $\pm$ 0.7
			40.00	42.84	101.0 $\pm$ 0.6
	B	0.08	10.00	9.39	93.2 $\pm$ 2.4
			20.00	20.70	103.1 $\pm$ 0.9
			40.00	38.56	96.2 $\pm$ 0.6
	C	0	10.00	10.12	101.2 $\pm$ 2.4
			20.00	20.46	102.3 $\pm$ 0.9
			40.00	39.36	98.4 $\pm$ 0.6
Glucose	A	12.22 * (12.17 $\pm$ 0.39)	10.00	21.69	97.6 $\pm$ 1.1
			20.00	32.06	99.5 $\pm$ 0.5
			40.00	52.27	100.1 $\pm$ 0.5
	B	8.96	10.00	17.58	92.7 $\pm$ 1.3
			20.00	28.58	98.7 $\pm$ 0.6
			40.00	47.44	96.9 $\pm$ 0.5
	C	3.72	10.00	12.92	94.2 $\pm$ 1.7
			20.00	22.79	96.1 $\pm$ 0.7
			40.00	42.01	96.1 $\pm$ 0.6
Fructose	A	49.58 * (49.11 $\pm$ 0.74)	10.00	59.70	100.2 $\pm$ 0.4
			20.00	69.86	100.4 $\pm$ 0.3
			40.00	90.21	100.7 $\pm$ 0.3
	B	39.52	10.00	49.55	100.1 $\pm$ 0.5
			20.00	61.10	102.6 $\pm$ 0.3
			40.00	83.18	104.6 $\pm$ 0.3
	C	23.60	10.00	34.00	101.2 $\pm$ 0.8
			20.00	54.03	100.8 $\pm$ 0.4
			40.00	64.94	102.1 $\pm$ 0.4
Glycerol	A	1.32	2.50	3.76	98.5 $\pm$ 2.1
			5.00	6.12	96.9 $\pm$ 1.3
			10.00	10.57	93.4 $\pm$ 0.8
	B	1.68	2.50	3.96	94.8 $\pm$ 2.4
			5.00	6.30	94.3 $\pm$ 1.5
			10.00	10.84	92.8 $\pm$ 0.9
	C	2.10	2.50	4.43	96.4 $\pm$ 2.4
			5.00	6.70	94.3 $\pm$ 1.6
			10.00	11.88	98.2 $\pm$ 1.0
Ethanol	A	13.56	15.41	26.97	93.2 $\pm$ 1.6
			30.83	42.31	95.3 $\pm$ 1.1
			61.66	73.99	98.4 $\pm$ 0.7
	B	25.74	15.41	42.16	102.4 $\pm$ 1.2
			30.83	58.50	103.4 $\pm$ 1.1
			61.66	87.86	100.5 $\pm$ 0.8
	C	26.67	15.41	39.61	94.2 $\pm$ 1.6
			30.83	53.56	93.1 $\pm$ 0.9
			61.66	87.86	99.5 $\pm$ 0.6
Sorbitol	A	4.10	2.50	6.78	102.8 $\pm$ 2.6
			5.00	9.11	100.1 $\pm$ 2.9
			10.00	13.86	98.3 $\pm$ 1.3
	B	4.06	2.50	6.76	103.1 $\pm$ 3.7
			5.00	9.29	102.5 $\pm$ 1.9
			10.00	14.26	101.4 $\pm$ 1.4
	C	4.05	2.50	6.77	103.3 $\pm$ 1.6
			5.00	9.37	103.5 $\pm$ 1.8
			10.00	14.44	102.8 $\pm$ 1.7

* Values obtained from enzymatic analysis.

glucose and sorbitol, but a cation-exchange resin column in calcium form has been shown to separate glucose, fructose, sucrose, sorbitol, glycerol and ethanol. The proposed procedure may be useful for controlling the evolution of the sugars and their transformation during the ripening of the apple. Minimal sample preparation, rapid quantitative analysis and good repeatability support this procedure as a good method for the monitoring of sugars, polyalcohols and ethanol in the course of fermentative processes of apple juice.

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