Articles

Chemometric Aspects of Sugar Profiles in Fruit Juices Using HPLC and GC

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The objective of this work is to determine the sugar profiles in commercial fruit juices, and to obtain chemometric characteristics. Sugar compositions of fruit juices were determined by HPLC-RID and GC-FID *via* methoxymation and trimethylsilylation with BSTFA. The appearance of multiple peaks in GC analysis for carbohydrates was disadvantageous as described in earlier literatures. Fructose, glucose, and sucrose were major carbohydrates in most fruit juices. Glucose/fructose ratios obtained by GC were lower than those by HPLC. Orange juices are similar to pineapple juices in the sugar profiles. However, grape juices are characterized by its lower or no detectable sucrose content. In addition, it was also found that unsweeten juices contained considerable level of sucrose. Chemometric technique such as principal components analysis was applied to provide an overview of the distinguishability of fruit juices based on HPLC or GC data. Principal components plot showed that different fruit juices grouped into distinct cluster. Principal components analysis was very useful in fruit juices industry for many aspects such as pattern recognition, detection of adulterants, and quality evaluation.

Introduction

Sugar is the major constituent of most fruit juices. Carbohydrates have been recognized as structural materials and sources of energy in biological world. In the area of human health, carbohydrates play various roles from human diet to cardiovascular disease, diabetes, and dental decay. In addition, biological aspects of bacterial infection, immunological protection, cell adhesion, and reproduction have been attracted a great interest.

Due to the importance of carbohydrates, their analytical methods have received a great deal of attention. Gas chromatography (GC)[1,2,3] has been used to separate carbohydrates employing several types of derivatization. Since the polar hydroxyl, amino, and carboxyl groups of carbohydrates make carbohydrates nonvolatile, the volatility of carbohydrates is greatly increased by derivatizations of these groups. Average detection limit is ng/ml level. A general difficulty of GC was the appearance of multiple peaks in the chromatogram due to the presence of tautomeric forms of reduced sugars. High performance liquid chromatography (HPLC)[4,5,6] for carbohydrates involves amine column or anion exchange column at either low or high pH. Generally carbohydrates do not absorb ultraviolet (UV) and visible radiation or fluorescence without suitable prior derivatization, because they possess neither chromophore nor fluophore. Some carbohydrates absorb near UV radiation in the region of 180-220 nm. Detection based on measurement of the absorption in this range is, however, nonselective. Measurement of refractive index (RI) is the direct method of detection for carbohydrates in HPLC. RI detector has sensitivities less than desired and estimate detection limit is 1 μg/mL. In a few cases, electrochemical detection is applied to readily oxidizable carbohydrates.

Previous study selected a number of fruits and fruit concentrates by GC to define the characteristic peaks associated with natural fruits as a critical index of adulteration in commercial products.[7,8] Hurst *et al.* included juices among variety of food samples in study for the characterization of individual carbohydrates in foods by using HPLC.[9] Deviations in sugar patterns have been used as evidence of adulteration in many fruit juices.[10,11] Adulteration of fruit juices can range from simple addition of sugar solutions acidified with organic acids to addition of cheaper, more available juices.

Chromatographic data were obtained for most common fruit juices but no chemometric attempt was made to distinguish sugar patterns related to different fruit juices. In a general way, chromatographic data may represent by a multivariate data matrix denoted by X which has n samples and p variables (each individual content of carbohydrates). Chemometric method such as principal components analysis (PCA) is useful for extracting potential informations from complex chromatographic profiles. PCA is used mainly to achieve a reduction of dimensionality, *i.e.*, to fit a j-dimensional subspace from the original p-variate ($p>j$) space of objects and permit a primary evaluation between category similarity. Recently, the application of chemometric methods for characterizing or classifying food products according to origin, quality, variety, type, or other features has already attracted considerable interest from researchers.[12,13,14] Several applications were also reported by Lee *et al.*[15,16,17,18]

The primary objective of this study is to determine the

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sugar profiles in commercial fruit juices by using GC and HPLC, and to obtain chemometric characteristics which will be particularly useful for detecting adulteration, quality evaluation and product formulation.

Experimental

Materials. Commercially available fruit juices listed in Table 1, supplied by different vendors, including five apple juices, eight orange juices, five grape juices, three pineapple juices, a carrot juice, and a coconut juice, were obtained from supermarket. Experimental standards of sugars were purchased from Sigma. Chromatographic grade acetonitrile were purchased from Merck. Water used throughout this study was purified by using E-pure water purification system following double distillation and deionized. Specific conductivity of this water was 18 megasiemens. Analytical grade o-methylhydroxylamine hydrochloride (MHA), N-o-bis-(TMS)-trifluoroacetamide (BSTFA), and phenyl-β-D-glucoside were obtained from Tokyo Kassei. Pyridine was purified by simple distillation prior to use.

HPLC procedure. Juice sample was centrifuged for 20 min. at 15,000 rpm, and then filtered with 0.45 μm membrane filter. This analytical sample was diluted with water if dilution was necessary. HPLC system consisted of a model LC-9A (Shimadzu) pump, Rheodyne 7125 injector with 20 μL sample loop, model 6-A RI detector (Shimadzu) and CR-6A integrator (Shimadzu). Analytical separation was achieved on a Carbohydrate analysis column (Waters, 3.9 mm × 300 mm) using a mobile phase of acetonitrile and water (83 : 17 v/v %) at a flow rate of 1.0 mL/min, and column temperature was kept at 35 °C in CTO-6A oven (Shimadzu). Retention time of each components was compared with that of authentic compound for identification. Standard solutions of xylose, fructose, glucose, sucrose, and maltose were prepared in mobile phase. Triplicate injections were made for each solution and the peak area was plotted against the corresponding concentration to obtain calibration curves.

Methoxymation and trimethylsilylation with BSTFA. Juice sample was centrifuged for 20 min. at 15,000 rpm, and then filtered with 0.45 μm membrane filter. This filtrate was evaporated to dryness by freeze-drying method by Senter *et al.*[19] or evaporated under reduced pressure by Zegota.[20]

Table 1. Physicochemical properties of fruit juices used in this study (Mean±R.S.D%)

Sample code	brand	pH	Total acidity mg acid*/100 mL	Density g/mL
	<Apple juice>			
A-1	Lotte EVE 100% (can)	3.62±0.13	31.65±0.99	1.047±0.024
A-2	Haitai fresh 100 100% (can)	3.56±0.95	31.65±0.52	1.050±0.046
A-3	Korea uri 100% (can)	3.83±0.25	26.16±0.61	1.046±0.015
A-4	Sunkist family 100% (jar)	3.30±0.38	49.81±0.64	1.049±0.034
A-5	Hi-C 100 gold 100% (jar)	3.67±0.13	31.53±1.74	1.048±0.024
	<Orange juice>			
O-1	Delmont unsweetened 100% (jar)	3.68±0.13	73.57±1.13	1.047±0.012
O-2	Delmont premium 100% (jar)	3.69±0.26	64.37±0.64	1.043±0.006
O-3	Sunkist family 100% (jar)	3.67±0.34	64.99±0.22	1.043±0.007
O-4	Sunkist refresh 100% (jar)	3.72±0.13	64.70±0.35	1.041±0.059
O-5	Korea modni 100% (jar)	3.62±0.23	72.72±0.39	1.043±0.002
O-6	Hi-C 100 100% (can)	3.54±0.27	75.57±0.42	1.047±0.003
O-7	Hi-C premium gold 100% (jar)	3.63±0.26	67.85±0.09	1.041±0.003
O-8	Haitai cecibong 100% (can)	3.77±0.25	51.81±0.12	1.046±0.008
	<Grape juice>			
G-1	Sunkist fresh 100 100% (can)	3.15±1.45	39.77±0.45	1.052±0.023
G-2	Lotte Eve 100% (can)	3.22±0.44	47.19±0.67	1.058±0.051
G-3	Hi-C 100 gold 100% (jar)	3.36±0.88	36.96±0.50	1.065±0.021
G-4	Delmont squash gold 50% (can)	2.87±0.59	44.25±0.42	1.045±0.010
G-5	Sunkist sweethome 30% (jar)			
	<Pineapple juice>			
P-1	Delmont tabong 50% (can)	3.42±0.36	50.75±0.53	1.052±0.009
P-2	Sunkist family 100% (can)			
P-3	Delmont squash gold 100% (can)	3.17±2.98	47.15±1.13	1.052±0.020
	<Carrot juice>			
CA-1	Gaya 100% (jar)			
	<Coconut juice>			
CO-1	Nuboon coconut meat 2% (can)			

*A; malic acid. O and P; citric acid. G; tartaric acid (n=3)

A portion of phenyl-β-D-glucoside (internal standard) and 0.5 mL of anhydrous pyridine solution of MHA (25 mg/mL) were added to 10 mg of dry sample. Then reaction vial was heated at 80 °C for 60 min. Subsequently BSTFA is added to the mixture, then reaction vial was stoppered and reheated. After it is cooled, an aliquot was submitted to GC.

GC procedure. GC analyses were performed on a model GC-14B (Shimadzu) equipped with a split/splitless capillary injector and flame ionization detector (FID). Analytical separation was achieved on a crosslinked methyl silicone gum fused silica capillary column (Ultra-1, 25 m in length, 0.32 mm i.d., 0.52 μm film thickness; Hewlett Packard). The gas flow rates were kept as follows: carrier gas (nitrogen), 1.1 mL/min; hydrogen, 38 mL/min; air, 460 mL/min. The amount injected was 0.1 μL and split ratio was 1 : 67. Temperatures of injector and FID were 300 °C. Column initial temperature was held at 180 °C for 1 min and then programmed to 290 °C at 5 °C/min, and held at 290 °C for 10 min. GC peak areas were integrated with CR-6A integrator (Shimadzu). Derivatized sample was also analysed on a QP-2000 GC-MS (Shimadzu) using methyl silicon capillary column (CBP-1, 25 m in length, 0.22 mm i.d., 0.25 μm thickness, Shimadzu) for identification of components at 70 eV of EI ionization. The GC retention time and mass fragmentation pattern of each component of sugars was compared with that of authentic compound for identification.

Principal components analysis. PCA was performed by using MVSAP (multivariate statistical analysis program, version 3.1) software which developed at our laboratory. Contents of fructose, glucose, and sucrose measured in n samples represented the values of p variables, and it made $n \times p$ matrix. From this matrix, variance-covariance matrix, eigen value, eigen vector, cumulative proportion, and principal components score for each sample were computed by personal computer.

Results and Discussion

HPLC-RID analyses. The separation of carbohydrates on unmodified silica gel column is almost impossible due to the interactions between the free silanol groups and the hydroxyl groups of carbohydrates. In this study, the choice of a aminopropylmethylsilyl bonded amorphous silica (Carbohydrate) column was based on the high efficiency in the separation of mono or disaccharides which were abundant in fruit juices. First mechanism involved in the separation of carbohydrates on chemically bonded amine phases is the distribution of analytes between the stationary phase and mobile phase. A second disputed mechanism proposes that retention is caused by the formation of hydrogen bonds between the hydroxyl groups of carbohydrates and the amino groups on the stationary phase.

Optimization of the separation on amine column leads to the use of 83 v/v % acetonitrile in water as mobile phase at the flow rate of 1.0 mL/min. Chromatogram and chromatographic parameters for standard mixture of xylose, fructose, glucose, sucrose, and maltose are given in Figure 1 and Table 2, respectively. Elution order was in good agreement with previous report by Doughty *et al.*[21] The correlation of peak areas with the concentrations of sugars studied, respectively, was linear in the range of 0.25-4 mg/mL, as shown in Figure 2. The correlation coefficients of the standard curve were better than 0.998. The detection limit, defined by a signal to noise ratio of 2 : 1 was less than 6.1 μg/mL. This result was in good agreement with previous data reported by McGinnis *et al.*[22] The reproducibility defined as the relative standard deviation of replicate analyses varied from 3.56% to 9.55%.

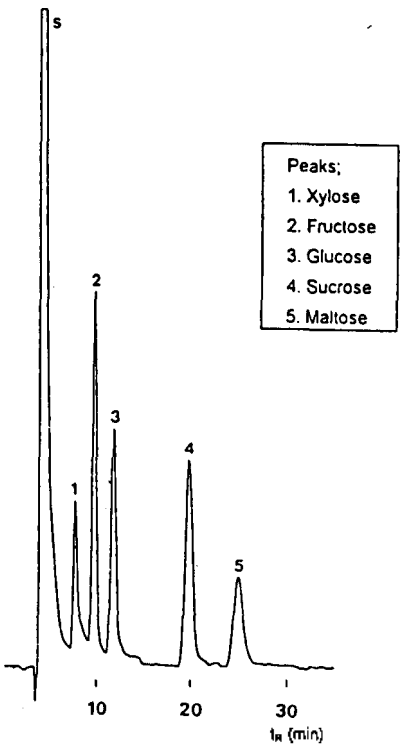

Figure 1. HPLC chromatogram of sugar standards. Carbohydrate column (Waters, 3.9 mm × 300 mm); acetonitrile/water (83 : 17); flow rate, 1.0 mL/min; column temperature, 35 °C; RI-detector, 8×10^{-6} RIU.

Table 2. *The liquid chromatographic parameters*

No	Sugars	t_R (min)	t_R' (min)	k'	α	Rs	N	H (mm)
1	Xylose	7.455	3.693	0.982	1.545	1.918	1926	15.576
2	Fructose	9.467	5.705	1.517	1.350	2.177	3106	9.659
3	Glucose	11.462	7.700	2.047	2.041	5.780	4553	6.589
4	Sucrose	19.475	15.713	4.177	1.324	3.537	5134	5.843
5	Maltose	24.570	20.804	5.531			5230	5.736

Sugar compositions of fruit juices determined by HPLC-RID. The chromatograms of sugars obtained from six different kinds of fruit juices are shown in Figure 3. The results of compositional determination based on the calibration curve are listed in Table 3.

Fruit juices have characteristic sugar compositions that are useful for evaluating product quality and authenticity. Fructose, glucose, and sucrose were major carbohydrates. Absence of a maltose peak reflects the advances in corn syrup technology with complete hydrolysis of starch to monosaccharides taking place and/or maltose being removed in the ion-exchange resin refining process.[10] Relative ratio

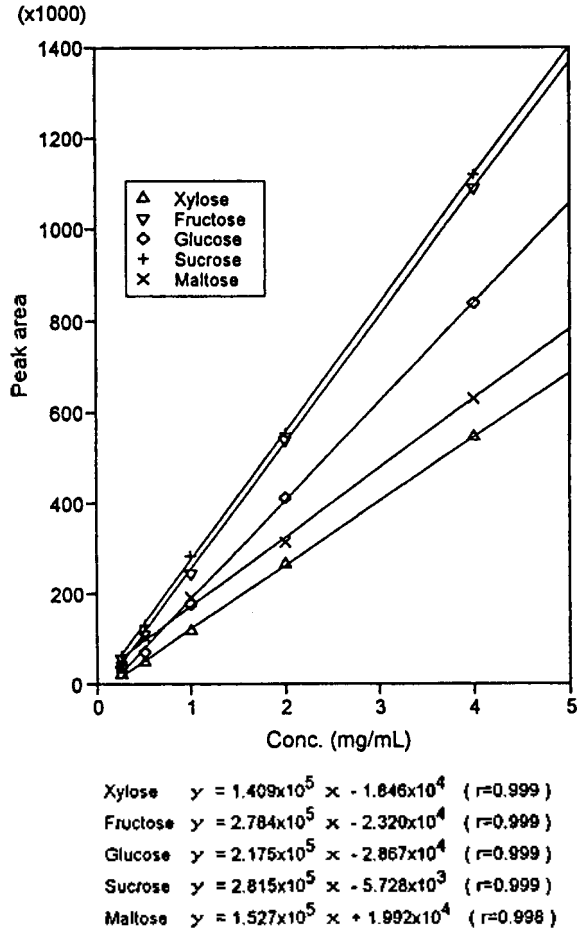

Figure 2. Calibration curves of sugar standards determined by HPLC-RID.

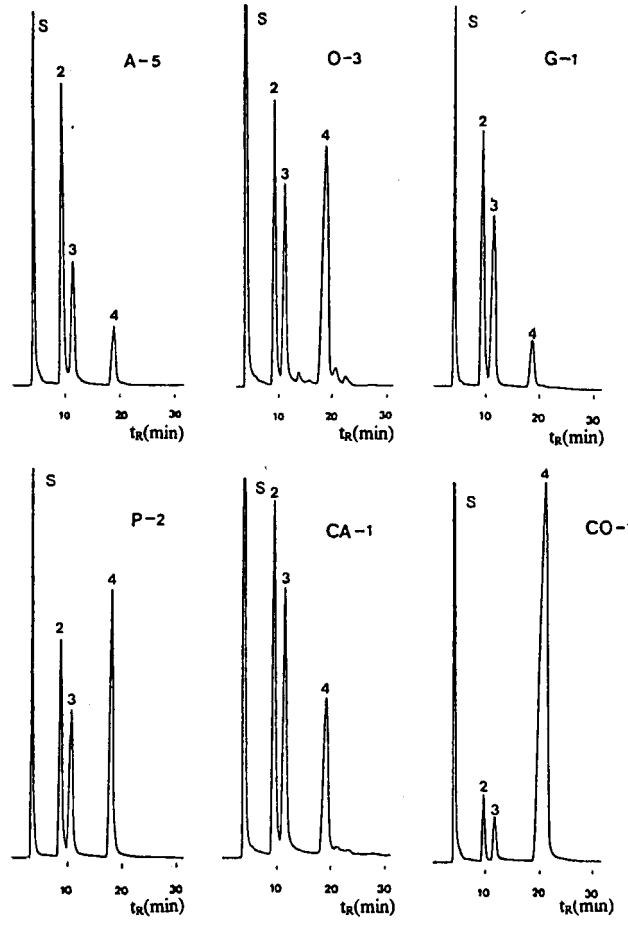

Figure 3. HPLC chromatograms of various fruit juices. Alphabetic sample codes and peak numbers correspond to those of Table 1 and Figure 1, respectively.

Table 3. Contents of sugars in fruit juices determined by HPLC-RID (g/100g)

Sample	Fructose[a]	Glucose[a]	Sucrose[a]	Total[b]	G/F ratio
A-1	6.263±0.197	4.248±0.249	1.468±0.136	11.979±0.582	0.678
A-2	5.832±0.436	4.182±0.409	1.206±0.010	11.220±0.855	0.717
A-3	4.889±0.257	3.254±0.111	1.543±0.018	9.686±0.386	0.666
A-4	6.706±0.395	5.021±0.217	1.035±0.028	12.762±0.640	0.749
A-5	5.905±0.214	4.092±0.183	1.800±0.110	11.797±0.507	0.693
O-1	2.807±0.115	3.277±0.163	3.461±0.043	9.545±0.321	1.167
O-2	3.100±0.572	3.572±0.672	4.146±0.435	10.818±1.680	1.155
O-3	2.944±0.418	3.377±0.379	4.935±0.583	11.256±1.380	1.147
O-4	2.655±0.181	2.901±0.039	3.360±0.244	8.916±0.464	1.093
O-5	2.881±0.140	3.292±0.023	2.850±0.112	9.023±0.275	1.143
O-6	2.662±0.150	3.221±0.033	3.143±0.571	9.026±0.574	1.210
O-7	2.664±0.204	2.837±0.052	3.517±0.102	9.018±0.358	1.065
G-1	6.562±0.047	6.948±0.135	1.208±0.053	14.718±0.235	1.059
G-2	7.029±0.066	7.303±0.195	0.253±0.061	14.585±0.322	1.039
G-3	7.144±0.118	7.368±0.190	0.571±0.125	15.083±0.433	1.031
G-4	4.931±0.308	4.039±0.142	ND	8.970±0.450	0.819
G-5	4.402±0.011	2.865±0.054	ND	7.267±0.065	0.651
P-2	2.813±0.097	3.481±0.080	6.181±0.030	12.475±0.207	1.237
P-3	3.920±0.078	4.676±0.442	2.676±0.425	11.272±0.945	1.193
CA-1	3.117±0.071	3.611±0.156	2.310±0.044	9.030±0.267	1.158
CO-1	0.790±0.043	0.781±0.065	10.835±0.720	12.406±0.828	0.989

[a] mean±s.d (n=3). [b] sum of fructose, glucose, and sucrose

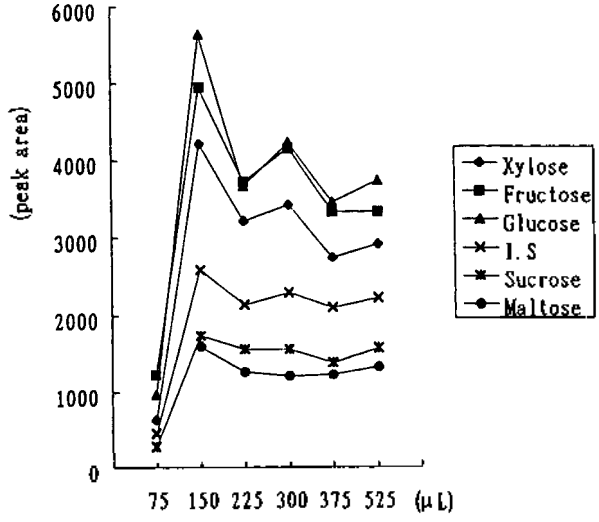

Figure 4. Influence of added amount of BSTFA on GC-FID response.

along with glucose: fructose (G/F) is useful index for distinguishing these fruit juices. G/F ratio obtained from pineapple juices were *ca* 1.20, and this experimental values was different from the value of 0.86 reported by Pilando *et al.*[10] Orange juices show similar profile with pineapple juices. However, a distinctly different profile for grape juices is characterized by its lower or no detectible sucrose content. This value is within the range of literature value by Swallow *et al.*[23] In this study, unsweeten juices exibit considerable level of sucrose.

GC-FID analyses. A modified methoxymation and trimethylsilylation described by Senter[19] for the preparation of derivatives was applied in this study. Derivatization was achieved by using freeze-dryed sample. However, it is impossible to derivatize when sample was evaporated under reduced pressure, because trace moisture was removed in-

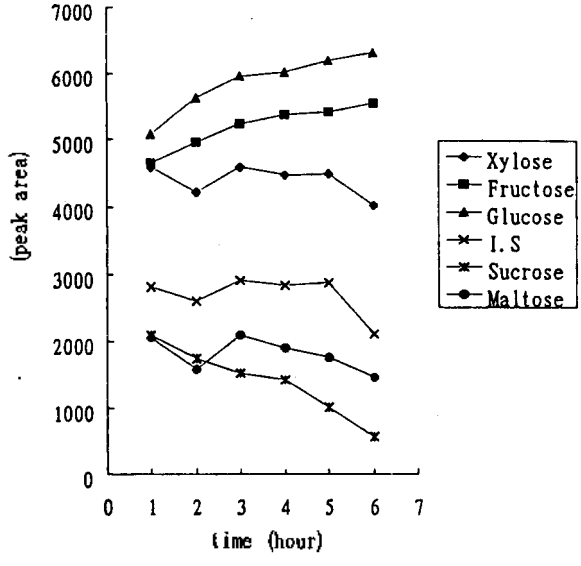

Figure 5. Influence of reheating time of derivatization on GC-FID response.

completely from the reaction mixture.

Influence of added amount of BSTFA on GC response was investigated. Each standard sugars (3 mg, respectively) and internal standard were treated with 5 mL of MHA-pyridine solution for 60 min at 80 °C. Then varied amount from 75 μL to 525 μL of BSTFA reagent was added. The best response was observed when 150 μL of BSTFA was added, as shown in Figure 4. Amount of added BSTFA was fixed as 150 μL for all measurement, influence of reheating time on GC response was tested. The best con-ditions were established when reheating time was 1 hr at 80 °C as can be observed from the results presented in Figure 5.

A variety of derivatizing methods have been used for the GC analysis of sugars and most of them are undesirable when ketoses are present. The main advantage of methoxime-TMS derivatives is applicable on ketoses. The use of BSTFA for the preparation of volatile derivatives for GC analysis was first described by Lane and Sweeley.[24] A notable advantage of using BSTFA derivatives from the possibility of injecting the reaction mixture directly for GC without detrimental effects to the analytical column.

Typical chromatogram of sugar standards was shown in Figure 6. The detection limits were 30.3-126 ng/mL and reproducibility was better than 4.94%.

As mentioned above, the appearance of multiple peaks in GC analysis for carbohydrates had disadvantage. Xylose and sucrose show single peak but fructose, glucose, and maltose show double peaks. Reduced sugars such as glucose exist in solution as an equilibrium mixture of forms

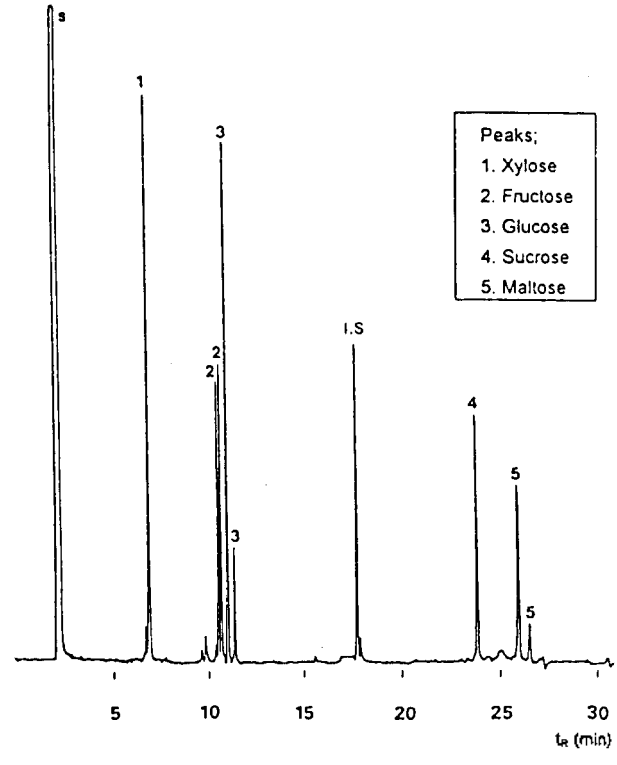

Figure 6. GC chromatogram of sugar standards. Ultra-1 column (Hewlett-Packard, 25 m × 0.32 mm i.d., 0.52 μm film thickness); oven temperature programming, 180 °C (1 min)-5 °C/min-290 °C (10 min); injector, 300 °C; FID, 300 °C; split ratio, 1 : 67. I.S., phenyl-β-D-glucoside.

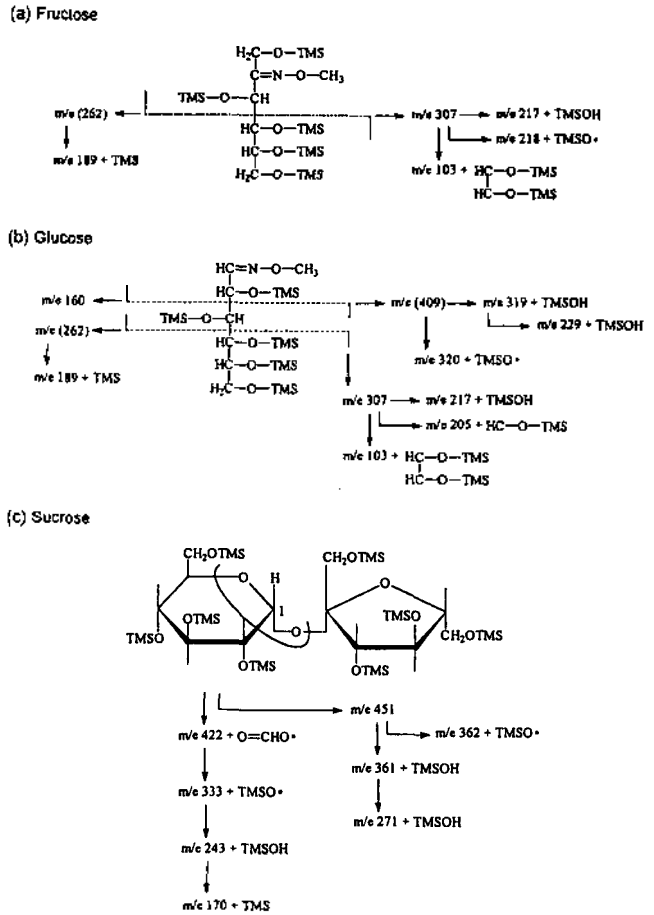

Figure 7. Possible fragmentation pattern of methoxymated and trimethylsilylated sugars.

known as anomers. Interconversion between the anomers occurs *via* the open chain form, with mutarotation resulting from the ready opening and closing of the hemiacetal ring. In order to minimize this interconversion, mild and rapid derivatization conditions are recommended.[25,26,27]

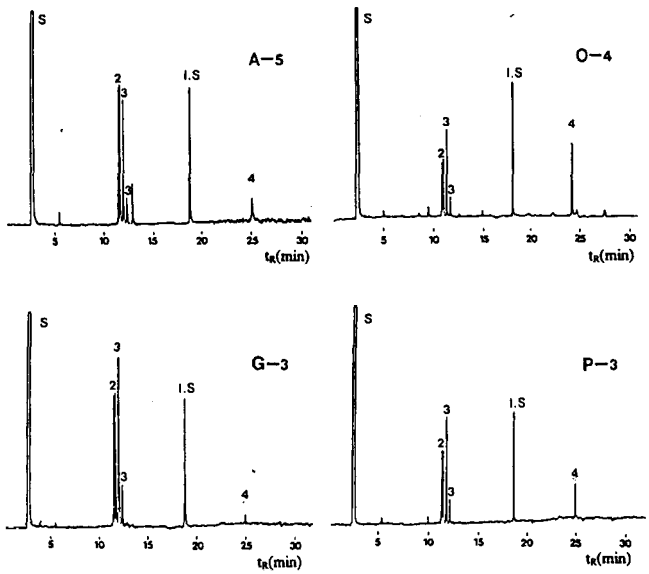

Figure 8. GC chromatograms of various fruit juices. Alphabetic sample codes and peak numbers correspond to those of Table 1 and Figure 6, respectively.

Along with $(CH_3)_3Si^+$, base peak at m/e 73 was observed in EI-mass spectra of fructose, glucose, and sucrose for an apple juice. Possible EI-fragmentation pattern of trimethylsilylated sugars were illustrated in Figure 7.

Sugar profiles of fruit juices determined by GC. Figure 8 shows sugar profiles of different fruit juices determined by GC. Fructose, glucose, and sucrose were major carbohydrate, however, absence of maltose peak were similar to HPLC data. Gas chromatograms of real juice sam-

Table 4. Contents of sugars in fruit juices determined by GC (g/100g)

Sample	Fructose[a]	Glucose[a]	Sucrose[a]	Total[b]	G/F ratio
A-1	6.154±0.075	3.400±0.016	0.703±0.058	10.257±0.149	0.552
A-2	5.051±0.031	3.424±0.001	0.880±0.527	9.355±0.063	0.678
A-3	6.110±0.023	3.648±0.005	1.246±0.031	11.004±0.059	0.597
A-4	6.895±0.125	4.670±0.287	0.917±0.064	12.482±0.476	0.677
A-5	5.969±0.007	3.992±0.100	1.469±0.040	11.430±0.147	0.669
O-1	2.363±0.458	2.252±0.474	3.046±0.705	7.661±1.637	0.953
O-2	2.747±0.067	2.483±0.126	3.809±0.022	9.309±0.215	0.094
O-3	2.098±0.214	1.812±0.198	2.899±0.005	6.809±0.417	0.864
O-4	2.679±0.118	2.368±0.158	3.680±0.889	8.727±1.165	0.884
O-5	3.730±0.118	2.855±0.112	3.480±1.046	10.065±1.276	0.765
O-6	2.286±0.032	1.926±0.028	3.371±0.223	7.583±0.293	0.843
O-7	2.627±0.091	2.162±0.072	3.461±0.199	8.250±0.362	0.823
G-1	8.309±0.057	6.923±0.081	0.655±0.007	15.887±0.145	0.833
G-2	6.681±0.032	4.785±0.039	ND	11.466±0.071	0.716
G-3	7.600±0.082	5.815±0.057	0.592±0.017	14.007±0.156	0.765
G-4	5.855±0.035	4.284±0.026	ND	10.139±0.061	0.732
P-1	6.180±0.056	4.544±0.045	0.677±0.138	11.397±0.239	0.735
P-3	4.716±0.181	4.138±0.268	3.139±0.155	11.993±0.604	0.877

[a] mean±s.d (n=2). [b] sum of fructose, glucose, and sucrose

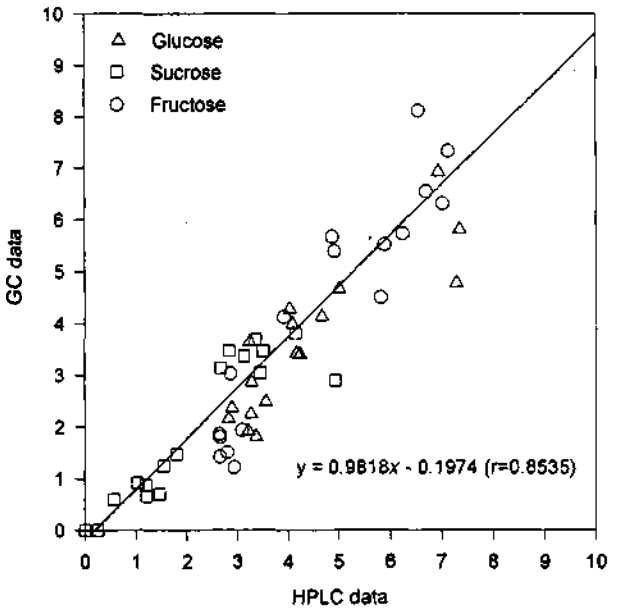

Figure 9. Correlation between HPLC data and GC data.

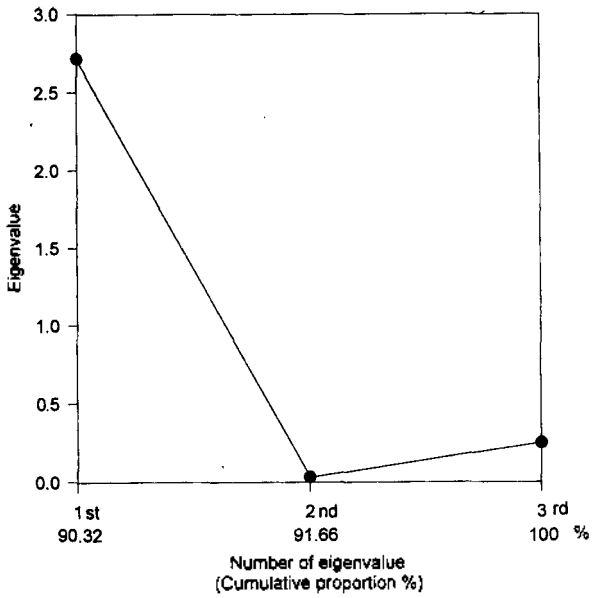

Figure 11. Scree graph for eigenvalues of sugar profiles in Table 4.

ples have shown double peaks of fructose and glucose. Sugar compositions determined by internal standard method are given in Table 4. G/F ratios by GC were lower than those of HPLC data. Correlation plot between HPLC data and GC data was shown in Figure 9.

Pricipal components analysis. PCA was applied to provide an overview of the distinguishability of fruit juices based on HPLC or GC data. The scree graphs, eigenvalue as a function of eigenvalue number, for HPLC and GC data show ideal trend, as shown in Figure 10 and 11. The cumulative proportions for second eigenvalue were 88.06% and 91.66%, respectively. These scree graphs suggest that the first two components for each samples might act as an adequate summary of the original data.

An important feature of the data is that a plot of first two principal components scores may reveal a tendency of the points to cluster. The plots in Figure 12 and 13 showed that different fruit juices formed into distinct cluster; each groups could be distinguished clearly. Two groups which contain orange and pineapple juices are clustered because of their similar pattern of sugar compositions. However, coconut or grape juices are located apart from orange juice group. Obviously these results suggest that PCA study is very useful in fruit juices industry for many aspects such as pattern recognition or category similarity, detection of

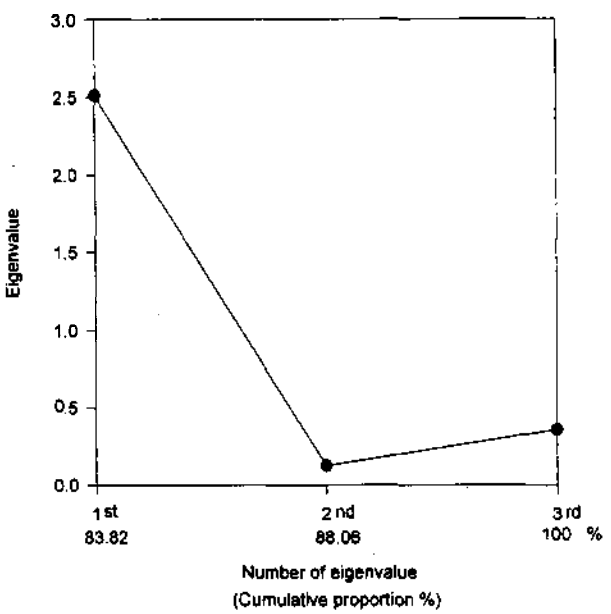

Figure 10. Scree graph for eigenvalues of sugar profiles in Table 3.

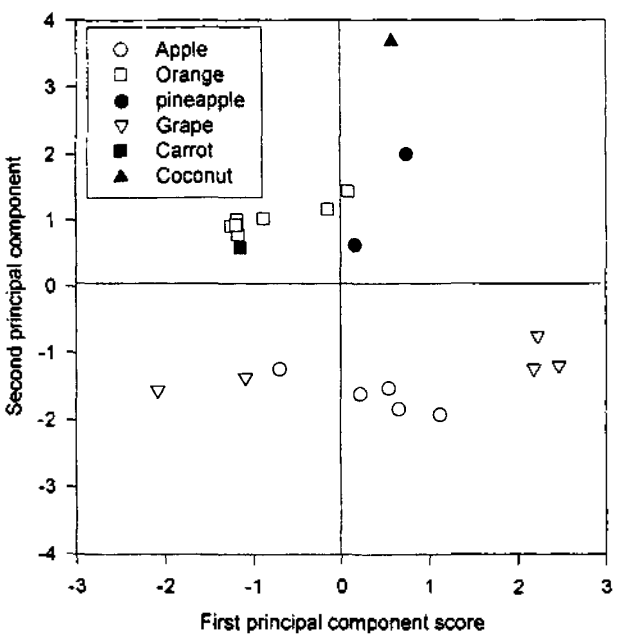

Figure 12. Principal components plot for sugar composition data of fruit juices determined by HPLC-RID.

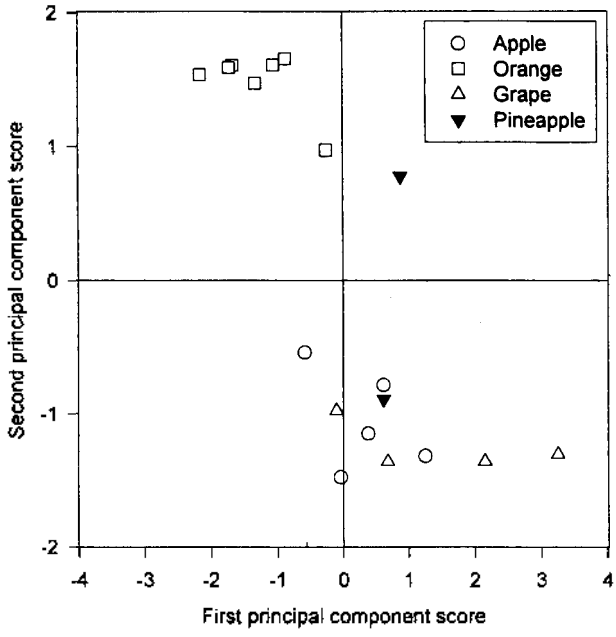

Figure 13. Principal components plot for sugar composition data of fruit juices determined by GC-FID.

adulterants, and quality evaluation.

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