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Simultaneous determination of organic acids and sugars in apples by gas–liquid chromatography

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ABSTRACT

A simple and rapid gas–liquid chromatographic method for the simultaneous determination of aliphatic mono-, di- and tricarboxylic acids, cyclic acids (shikimic + quinic), aromatic acids and sugars, all as trimethylsilyl derivatives in a single solution, was developed. Optimum conditions for the simultaneous derivatization and for the quantitative evaluation of organic acids and sugars were established. Reproducibility data showed that 0.25–1 μg of the characteristic acids of apples in the presence of a 700–2500-fold excess of sugars can be determined with a relative standard deviation (R.S.D.) of 10.7% or less. The main components (malic acid, fructose, glucose and sucrose) were determined with an R.S.D. of 4.0% or less.

INTRODUCTION

Usually carboxylic acids and sugars, present in fruit juices and related samples, have been determined separately, applying different conditions. Suggested methods are mainly enzymatic [1–4] and chromatographic procedures [5–14], commonly used in parallel. Recently, some attention [9,10,12–14] has been paid to their simultaneous determination: the major organic acids and sugars in apple juice and cider [9] and in cucumber fermentations [10] have been determined by high-performance liquid chromatography (HPLC), and in grape musts [12], propolis [13] and birch sap [14] as trimethylsilyl (TMS) derivatives by gas–liquid chromatography (GLC). However, with one exception [14], the components to be measured were present in nearly the same concentration range.

In this paper we describe a technique, based on trimethylsilylation and GLC, which makes possible the simultaneous determination of sugars and acids. This method has been applied to the determination of the main components of apple and its products (apple juice, clarified apple juice, concentrate).

EXPERIMENTAL

Materials and reagents were of analytical-reagent grade from Reanal (Budapest, Hungary), Fluka (Buchs, Switzerland) and Serva (Heidelberg, F.R.G.).

Golden Delicious apple (sample 1), apple juice (sample 2), clarified apple juice (sample 3) and concentrate (sample 4) were obtained from the Nagykőrös Canning Factory (Nagykőrös, Hungary), henceforth referred to as the Factory. Sample 1 was

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made in our laboratory by pressing ground apple through gauze, and was analysed without further treating. Sample 2 was prepared in the Factory: ground apples were treated with enzymes, using Phylazim C (1000 g per 1000 kg of apple) and Pectinol M17 (200 g per 1000 kg of apple), both for 15–20 min. Sample 3 was the clarified version (Bentonite, Needer, IBS) of sample 2. Sample 4 was obtained from sample 3, concentrating it until the dry matter content was 68–70% (w/w).

Preparation of the TMS oxime of TMS derivatives

Various amounts of organic acids ($7.5 \cdot 10^{-5}$–$3.0 \cdot 10^{-4}$ g) and the same amounts of sugars (0.12 g of fructose, 0.045 g of glucose, 0.045 g of sucrose) in model solutions, or the corresponding amounts of samples 1–4 (1.5–2 g), were evaporated to dryness in a rotary evaporator at 50–60°C. The dehydrated residues were treated with 3.0 cm^3 of pyridine (containing 1.25 g of hydroxylamine hydrochloride per 100 cm^3) and were heated for 30 min at 75°C. The cooled samples were then trimethylsilylated with a mixture of 5.5 cm^3 of hexamethyldisilasane (HMDS) and 0.6 cm^3 of trifluoroacetic acid (TFAA) in 15-cm^3 Reacti-vials for 60 min at 100°C. Thereafter the filtered solutions were evaporated to 1.5–1.8 cm^3 in a rotary evaporator at 50–60°C. The residues were transferred quantitatively to 3-cm^3 Reacti-vials with 0.9 cm^3 of HMDS and 0.1 cm^3 of TFAA and diluted to 3-cm^3 volume with dichloromethane. Note: because in apple samples the sugars are present at greatly higher concentrations than acids, usually the sugar contents were calculated from a 50-fold diluted stock solution.

Separation of TMS oxime or TMS derivatives

The gas chromatograph used was a Chromatron Model G.C.H.F. 18.3 instrument equipped with a flame ionization detector and stainless-steel columns (3 m × 4 mm I.D.) were used. The packing material was 15% Dexsil GC 300 on 80–100-mesh Chromosorb W AW DMCS purchased from Applied Science Labs. (State College, PA, U.S.A.).

The temperatures of the injection and detector ports were 380 and 400°C, respectively. With a temperature programme from 60 to 360°C at 12°C/min and a hold at 360°C for 5 min, it required 30 min to elute the TMS oxime and TMS derivatives of organic acids and sugars. The flow-rate of nitrogen was 60 cm^3/min.

RESULTS AND DISCUSSION

Derivatization and GLC conditions

Derivatization. In order to achieve quantitative derivatization simultaneously with organic acids and sugars (without any side-reactions), further studies were needed to establish the optimum reaction time and temperature for trimethylsilylation and the appropriate GLC parameters.

As is known [15], the common parameters for both oximation and trimethylsilylation of sugars are a reaction time of 30–60 min and a temperature of 70–75°C. These conditions proved to be satisfactory for the quantitative silylation of the aliphatic series of acids (C_1–C_{22} fatty acids, C_2–C_{16} dicarboxylic acids), but not for the various aromatic and phenolic acids.

Hence a systematic study was carried out with sugars (fructose, glucose, su-

crose) and with the most difficult to derivatize organic acids such as ferulic, caffeic and chlorogenic acid (henceforth referred to as phenolic acids) as a function of the trimethylsilylation temperature (75, 100 and 125°C) and reaction time (10, 30, 60 and 120 min).

The results obtained showed that following to our derivatization protocol, even the sensitive sugar derivatives remain intact under the most severe conditions (125°C, 120 min). For the trimethylsilylation of phenolic acids, in order for them to be eluted in a single peak they require a temperature of 100°C and a reaction time of 60 min, or 125°C and 20 min; with a shorter reaction time (100°C, 30 min) or at lower temperature (75°C, 60 min), all three acids emerge in at least two peaks, probably owing to their partially derivatized hydroxyl group(s).

The proposed method is the first for the simultaneous determination of sugars and acids in the same stock solution in extremely different concentration ranges, owing to the important discovery that the silylated solutions of acids and sugars can be evaporated, without any irreversible alterations to the derivatized compounds, to one fifth of their original volume. In order to determine also the minor components of apple samples, 1–2 g of the 13–15% dry matter-containing juice are evaporated and derivatized, with a proportionately increased amount of reagents (see Experimental).

GLC. With a view to establishing the optimum GLC conditions, 2- and 3-m packed columns coated with 3% SE-30, 3% SP 2250 and 15% Dexsil 300 GC were tested. It was found that the 3% SE-30-coated columns gave an unsatisfactory resolution of acids and on the 3% SP 2250 columns citric acid could not be separated from fructose. Hence, as shown repeatedly in the past [15–18], 15% Dexsil 300 GC as support material furnished the best separating conditions also for the major sugars and characteristic acids of apples (it should be noted that this applies only with packed columns).

Reproducibility study

The reproducibility and the usefulness of the proposed method were shown by the results with model solutions (Table I, Fig. 1a) and by the composition of the Golden Delicious apple juice samples (Table II, Fig. 1b). The reproducibility measurements showed a relative standard deviation (R.S.D.) of $\leqslant 10.7\%$ for minor acids and $\leqslant 4.0\%$ for the main components such as malic acid and sugars.

Composition of apple samples

The sugar and acid contents of crude apple juices (samples 1 and 2), prepared in our laboratory (sample 1) and processed in the Factory (sample 2), and the composition of the clarified (sample 3) and concentrated (sample 4) versions of the Factory-made juice (sample 2) were measured as TMS derivatives (Table II).

Before the comparative evaluation of the sugar and acid contents of samples 1–4, it should be noted that in this connection (different places of preparation, clarification, concentration) no literature data were available. However, a number of publications [1–13,19] have dealt with differences that can be attributed to the variety, geographic origin, storage history, etc., of apple (juice) samples. Thus, in addition to comparing our results achieved with samples 1–4 with each other, the composition of sample 1 was also compared with the results of Lee and Wrolstad [19] obtained with laboratory-prepared juices of Golden Delicious apples.

TABLE I

REPRODUCIBILITY OF THE SIMULTANEOUS DETERMINATION OF VARIOUS AMOUNTS (a–c) OF ORGANIC ACIDS AND SUGARS AS TMS OXIME OR TMS DERIVATIVES

Prepared at 100°C for 60 min (A values) or 125°C for 20 min (B values). The test letters (a–c) identify the amounts of components injected: 0.25 μg (a_1, a_2), 0.5 μg (b_1, b_2), 1.0 μg (c_1, c_2) of organic acids of each [except chlorogenic acid: 0.85 μg (a_1, a_2), 1.70 μg (b_1, b_2) and 3.40 μg (c_1, c_2)] and 400 μg of fructose, 150 μg of glucose and 150 μg of sucrose in all six tests (a_1–c_2). Data in tests a_1, a_2, b_1, b_2 and c_1, c_2 were obtained from parallel derivatization procedures. Means of twelve tests [(A)a_1–c_2; (B)a_1–c_2)].

Component	Integrator units equivalent to 1 μg of substance												Mean	S.D.	S.D. (%)
	A						B								
	a_1	a_2	b_1	b_2	c_1	c_2	a_1	a_2	b_1	b_2	c_1	c_2			
Lactic acid	1080	1131	1142	1137	967	979	1039	1040	1002	1051	1248	1047	1072	80.6	7.5
Oxalic acid	1100	1075	904	885	1005	906	(810)[a]	(750)	950	913	1090	938	977	84.0	8.6
Sorbic acid	772	695	673	619	675	715	682	655	679	754	740	652	693	45.0	6.5
Benzoic acid	1390	1243	1210	1226	1035	(946)	(902)	1136	1240	1274	1219	993	1197	115.0	9.6
Succinic acid	971	875	910	882	941	935	(736)	(801)	914	989	859	909	919	41.4	4.5
Fumaric acid	1067	910	1236	1113	947	1113	–	–	–	–	–	–	1069	114.9	10.7
Malic acid	993	1050	978	951	1029	933	1005	1029	1046	1022	1035	956	1002	39.7	4.0
Tartaric acid	1200	1210	1093	1289	1089	1151	1168	1161	1115	1131	1191	1183	1158	43.2	3.7
Pimelic acid	900	1012	925	917	945	951	949	943	907	919	969	961	942	31.0	3.3
Shikimic acid	1106	1067	1290	1197	1111	1232	1167	1054	1246	1147	1150	1198	1164	72.4	6.2
Citric + quinic acids	854	897	815	847	851	930	921	(795)	867	832	826	951	872	45.8	5.3
Fructose	1096	1032	1019	1078	1034	1072	994	1023	1050	1023	979	1103	1042	38.8	3.7
Glucose	1074	1117	1093	1130	1052	1073	1051	1098	1129	1031	1078	1125	1088	33.3	3.1
C_{16} alkanoic + alkenoic acids	566	588	589	648	581	645	601	599	563	571	622	576	596	28.9	4.8
Caffeic + ferulic acids	565	543	571	545	548	542	564	568	586	569	545	562	559	14.1	2.5
C_{18} alkanoic + alkenoic acids	489	449	496	453	455	483	476	478	471	472	454	489	472	16.0	3.4
Sucrose	572	567	626	568	605	(666)	597	608	584	574	557	606	588	21.9	3.7
Chlorogenic acid	68	65	65	60	57	69	66	58	70	58	62	73	64	5.3	8.2

[a] Numbers in parentheses have been omitted from calculations.

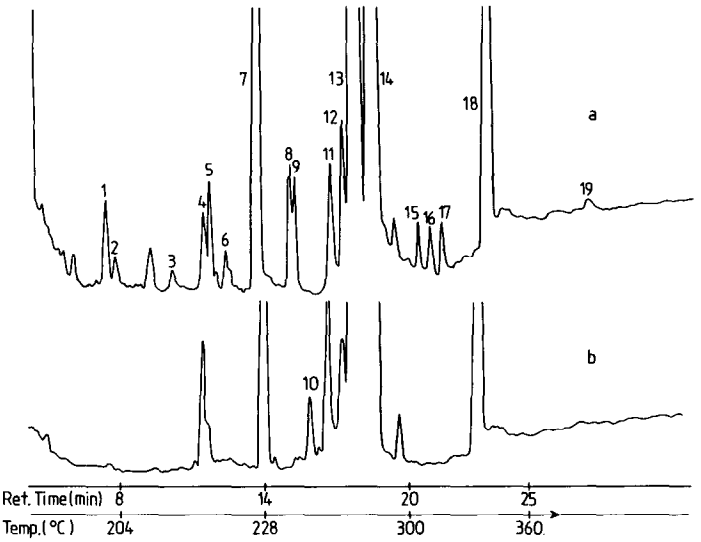

Fig. 1. Chromatograms of TMS and/or TMS oxime derivatives of acids and sugars obtained with (a) a model solution and (b) an apple sample (sample 2). Peaks: 1 = lactic acid; 2 = oxalic acid; 3 = sorbic acid; 4 = benzoic acid; 5 = succinic acid; 6 = fumaric acid; 7 = malic acid; 8 = tartaric acid; 9 = pimelic acid, 10 = arabinose, 11 = shikimic acid + rhamnose, 12 = citric + quinic acids, 13 = fructose, 14 = glucose, 15 = C_{16} fatty acids, 16 = caffeic + ferulic acids, 17 = C_{18} fatty acids, 18 = sucrose, 19 = chlorogenic acid.

As can be seen, the main differences in the compositions of samples 1–4 can be related to the place of preparation (sample 2, increased lactic acid and decreased rhamnose and sucrose contents), probably owing to the fact that in the Factory the bacteria for lactic acid fermentation are immediately available.

Smaller changes can be observed during the clarification procedure (sample 3, decreased oxalic and malic acid contents) and/or as a result of the concentration step (sample 4, decreased lactic acid, arabinose, fructose and glucose contents).

The basic point in the comparison of the composition of sample 1 with the acid/sugar contents of three other Golden Delicious apple juices (Golden Delicious '81 WA, Golden Delicious '82 WA and Golden Delicious Mexico, *i.e.*, varieties 1–3, henceforth referred to as v_1, v_2, v_3) could be[a] their very similar total malic acid contents (samples 1 = 4.35 g per 1000 g; $v_1 = 4.07$, $v_2 = 5.33$ and $v_3 = 4.61$ g/l). The fructose, glucose and sucrose contents of samples 1, v_1, v_2 and v_3, which in that order were fructose = 6.4 per 100 g, 8.61, 7.88 and 7.26 g per 100 ml, glucose = 2.5 g per 100 g, 3.64, 2.75 and 2.55 g per 100 ml and sucrose = 2.3 g per 100 g, 0.20, 1.53 and 2.96 g per 100 ml, were found to be much more variety-dependent than the total malic acid contents.

Similar results were obtained in the comparison of the fumaric acid (sample 1 = 1.3 mg per 100 g, $v_1 = 0.01$, $v_2 = 0.04$ and $v_3 = 0.17$ mg per 100 ml), quinic + citric

[a] As our values were normalized to 13.5% (w/w) dry matter contents whereas those of Lee and Wrolstad [19] were normalized to 12.5°C Brix.

TABLE II

SUGAR AND ORGANIC ACID COMPOSITION OF THE GOLDEN DELICIOUS APPLE SAMPLES (SAMPLES 1–4) MEASURED AS TMS OXIME OR TMS DERIVATIVES

Details as in Table I. Samples 1–4 were prepared as described under Experimental.

Compound	Sample No.			
	1	2	3	4
Carboxylic acids				
Lactic	6.4	7.9	7.7	7.3
Oxalic	0.53	0.59	0.49	0.49
Sorbic	0.70	0.68	0.70	0.68
Succinic	0.42	0.42	0.42	0.42
Fumaric	1.3	1.5	1.6	1.5
Malic	435	432	420	397
Tartaric	5.0	4.6	4.3	4.3
Pimelic	2.9	3.1	2.9	2.8
Citric + quinic	66	70	60	61
C_{16} alkanoic + alkenoic	1.7	1.9	1.8	1.8
C_{18} alkanoic + alkenoic	4.9	5.1	5.4	5.1
Caffeic + ferulic	0.29	0.29	0.29	0.29
Chlorogenic	4.0	4.2	5.2	4.5
Total amount, mg per 100 g sample[a]	525.1	532.3	507.4	487.2
Sugars				
Arabinose	51	44	48	37
Rhamnose[b]	52	35	35	37
Glucose	2.5	2.6	2.6	2.3
Fructose	6.4	6.3	6.4	5.6
Sucrose	2.3	1.8	1.7	1.7
Total amount, g per 100 g sample[a]	11.3	10.8	10.8	10.4

[a] Expressed on the basis of a dry matter content of 13.5% (w/w). The measured dry matter contents of samples 1–4 were (1) 15.4%, (2) 13.8%, (3) 13.3% and (4) 78.0%.

[b] Calculated as rhamnose, also containing some shikimic acid.

acids (sample 1 = 66 mg per 100 g, $v_1 = 236$, $v_2 = 186$ and $v_3 = 58$ mg/ml) and chlorogenic acid contents (sample 1 = 4.0 mg per 100 g, $v_1 = 3.15$, $v_2 = 3.53$ and $v_3 = 63.2$ mg per 100 ml).

In this paper both the general applicability with a study of model solutions (Table I) and a particular application with the analysis of apple samples (Table II) have been presented. The extension of the method to show its usefulness in the analysis of other natural matrices is in progress.

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REFERENCES

1 N. Gorin, *Z. Lebensm.-Unters.-Forsch.*, 167 (1978) 327; 168 (1978) 11.
2 H. J. Bielig and H. Gründing, *Flüss. Obst*, 52 (1985) 308.
3 M. Eckert, G. Baumann and K. Gierschner, *Flüss. Obst*, 54 (1987) 134.
4 C. H. Assoland-Vinet, G. Bardeletti and P. R. Coluet, *Anal. Lett.*, 20 (1987) 513.
5 A. R. Brause and J. M. Raterman, *J. Assoc. Off. Anal. Chem.*, 65 (1982) 846.
6 J. Zyren and E. R. Elkins, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 672.
7 E. D. Coppola and M. S. Starr, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 5.
8 D. B. Gomis, M. J. M. Gutierrez, M. D. G. Alvarez and J. J. M. Alonso, *Chromatographia*, 25 (1988) 1054.
9 D. B. Gomis, M. D. G. Alvarez, J. J. M. Alonso and A. N. Vallina, *Chromatographia*, 25 (1988) 701.
10 M. J. Lazaro, E. Carbonell, M. C. Aristoy, J. Safon and M. Rodrogo, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 52.
11 F. Tarrach and K. Herrmann, *Z. Lebensm.-Unters.-Forsch.*, 184 (1987) 38.
12 J. E. Marcy and D. E. Carroll, *Am. J. Enol. Vitic.*, 33 (1982) 176.
13 W. Maciejewicz, M. Daniewski and Z. Mielniczuk, *Chem. Anal. (Warsaw)*, 29 (1984) 421.
14 H. Kallio, S. Ahtonear, J. Paulo and R. R. Linko, *J. Food Sci.*, 50 (1985) 266.
15 C. F. Poole, in K. Blau and G. King (Editors), *Handbook of Derivatives for Chromatography*, Heijden and Son, London, 1978, pp. 152–194.
16 I. Molnár-Perl and M. Pintér-Szakács, *J. Chromatogr.*, 216 (1981) 219.
17 I. Molnár-Perl and M. Pintér-Szakács, *J. Chromatogr.*, 365 (1986) 171.
18 M. Morvai, M. Pintér-Szakács and I. Molnár-Perl, *J. Chromatogr.*, 446 (1988) 237.
19 H. S. Lee and R. E. Wrolstad, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 789.