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Chromatographic Profile of Carbohydrates in Commercial Soluble Coffees

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Pure soluble coffee, irrespective of extraction conditions, contains maximum levels of ca. 0.3% total xylose and sucrose, no maltose, and about 2% total glucose. Some commercial soluble coffees have been found to contain one or more of these sugars at higher levels. Elevated levels of total xylose are explained by coextraction with coffee husks/parchment. In this case, the level of free fructose and glucose distinguishes whether unroasted or roasted husks/parchments have been added. Elevated levels of maltose and total glucose indicate the addition of maltodextrins. Elevated levels of sucrose and total glucose show the addition of (caramelized) sugar.

The International Coffee Organization (ICO), an intergovernmental body formed by the coffee producer and consumer countries in close cooperation with the United Nations, defines "green coffee" as "all coffee in the naked bean form before roasting", "roasted coffee" as "green coffee roasted to any degree", and "soluble coffee" as "dried water-soluble solids derived from roasted coffee" (ICO, 1983). Other international bodies, such as the International Standard Organization (ISO), have given equivalent definitions and codified the impurities or "defects" such as wood, sticks, husks, parchment, or whole cherries, which may be present (ISO, 1984). Most countries have stated the maximum amount of defects tolerated in commercial coffee (Jobin, 1982). In applying these standards, visual defect counting is traditionally used to assess the purity of green beans, but this visual approach is obviously inappropriate for soluble coffees.

We have thus developed an analytical method based on screening the carbohydrate profile of instant coffee. The broad base of information obtained not only defines the normal composition of pure soluble coffees coming from a variety of coffee types and processing conditions but also can be used for characterizing the nature of various adulterants or additives occasionally found in these products.

While there have been numerous publications concerning the carbohydrate content in green and roasted coffees, only a few publications give quantitative data, with somewhat conflicting ranges, for the individual carbohydrates of soluble coffee (Streuli, 1970; Thaler, 1957; Pictet, 1975). Arabinose is indicated as the main free sugar (0.4–2.5%), followed by galactose (0.1–1.0%) and mannose (0.2–0.9%). Fructose and glucose were found to be less important (0–0.5%). Only traces of ribose and xylose are reported (Kroplien, 1974). Surprisingly, Trugo and Macrae (1982, 1985) found in a few cases appreciable amounts of free fructose and free glucose. Very little quantitative information is available on the total individual carbohydrates (free sugars plus sugars bound in large molecules): Pictet (1975) found in one sample 16.6% galactose, 12.2% mannose, 4.9% arabinose, and 1.4% glucose; Thaler (1957) quantified by paper chromatography the monosaccharides present in a sulfuric acid hydrolysate and found larger amounts of mannose and galactose and smaller amounts of glucose and arabinose. Only traces of xylose have been found by the various authors.

In the present work, we have established the carbohydrate profiles of many hundreds of soluble coffee samples. This has allowed us to differentiate pure soluble coffee products from adulterated products. It is shown that the values for both free and total sugar contents must be used in order to achieve this and to obtain information on the nature of the adulterants.

METHODS AND MATERIALS

HPLC of Sugars. *Apparatus.* An HPLC system, consisting of a Spectra-Physics SP-1800 (Spectra-Physics Inc., San José, CA), a Kratos URS 051 postcolumn derivatization (Kratos Inc., Ramsey, NJ), a Spectra-Physics SP-8773 XR UV detector, and an HP 1000 integrator, was used. Silica columns (SSMP, Spheri-5 silica 5 μm; Brownlee-Labs, Santa Clara, CA) were used for all analyses. These were prepared with a Supelco saturator with a 18-μm silica phase (Supelco Inc., Bellefonte, CA). The precolumn (Brownlee-Labs SS-GU) was also filled with silica.

Reagents. HPLC-grade acetonitrile came from Rowil Chemicals (Shepshed, England). For column preparation, a modified amine was obtained from Prof. Aitzetmüller (Natec, Hamburg, Germany). Tetrazolium Blue for the postcolumn reaction of carbohydrates was supplied by Sigma Chemical Co. (St. Louis, MO). The Carrez solution was prepared by mixing a solution of $K_4Fe(CN)_6 \cdot 3H_2O$, 35.9 g/L in distilled water, and a solution of $ZnSO_4 \cdot 7H_2O$, 71.9 g/L in distilled water (50/50, v/v).

Preparation of the Column. After being rinsed with acetonitrile, the precolumn and the column were impregnated with the modified amine solution (Aitzetmüller, 1980). In order to perform an efficient operation, 215 mg of modified amine in 400 mL of acetonitrile and 100 mL of water was recirculated for 15 h at 2 mL/min and at ambient temperature. The mixture was con-

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Table I. Free and Total Carbohydrate Contents of Industrial Soluble Coffees Made from Arabica and Robusta Roasted Coffees (% Dmb)

	free carbohydrate					total carbohydrate				
sample	Ara	Fru	Man	Glu	Gal	Xyl	Ara	Man	Glu	Gal
arabica (Santos NY 4-5)	2.20	0.21	0.29	0.17	0.53	0.19	5.01	10.20	1.21	19.40
arabica (Santos NY 4-5)	1.50	0.31	0.55	0.21	0.75	0.27	3.88	13.70	1.52	18.00
robusta (Ivory Coast IV)	1.26	0.04	0.18	0.03	0.33	0.13	4.16	14.00	0.56	16.50
arabica–robusta (15:85)	0.96	0.06	0.16	0.04	0.31	0.10	3.99	14.00	0.56	18.80
arabica–robusta (15:85)	1.18	0.13	0.47	0.07	0.52	0.17	3.51	16.30	0.73	18.10

tinuously degassed with a small flow of helium. The saturator was then removed, and the precolumn was replaced by a new one conditioned with pure acetonitrile. As proposed by Porsch (1982), the stationary phase was lowered to pH 6 with a phosphate solution (1.518 g of $NaH_2PO_4 \cdot H_2O$ in 1 L of distilled water, mixed with pure acetonitrile in 20/80 (w/w) proportions). This conditioning operation was at 38 °C with a flow rate of 2 mL/min.

Sample Preparation. *Standard Solution of Sugars.* A solution containing the following percentages of individual sugars was prepared in distilled water: xylose, 0.0035%; arabinose, 0.0130%; fructose, 0.0050%; mannose, 0.0340%; glucose, 0.0070%; galactose 0.0500%. Before injection, this solution was diluted 10 times with a 9/1 v/v mixture of water and acetonitrile.

Instant Coffee Solution for Free Sugar Analysis. One-hundred milligrams of instant coffee was dissolved in 100 mL of distilled water. This solution was cleaned by passing it through a Sep-Pack C18 cartridge and was then used directly for HPLC analysis.

Instant Coffee Solution for Total Sugar Analysis. To transform polysaccharides into free monosaccharides, the following hydrolysis conditions were used. One-hundred fifty milligrams of instant coffee was refluxed for 4 h in 135 mL of 1 N sulfuric acid. After cooling, 31.25 mL of Carrez solution was added and the pH adjusted to 2.5 with 11.5 mL of 10 N NaOH followed by filtration through a 597 1/2 prefolded-paper filter, discarding the first 20 mL. Before injection, this solution was diluted 5-fold in distilled water. Under these hydrolysis conditions, xylose, arabinose, mannose, glucose, and galactose are liberated with a recovery yield of 95% or higher.

Analytical Conditions. *Injection.* Ten-microliter portions of each solution to be analyzed were injected.

Separation. This was performed at 38 °C at a flow rate of 0.8 mL/min with 80/20 (w/w) acetonitrile/phosphate solution as eluant as previously described.

Detection. Postcolumn derivatization was performed with a Tetrazolium Blue solution. So as to use this in the presence of phosphate, 2 g of reagent was dissolved in 2 L of 0.02 N NaOH and the resultant mixture filtered. The temperature of the postcolumn system was 80 °C, and detection was at 530 nm.

Quantification. The standard solution of sugars was used as an external reference for quantification.

Thin-Layer Chromatography. The TLC plates used were silica gel 60, 10 × 20 cm (Merck 5641, Merck, Darmstadt, Germany), with a mixture of 1-butanol/methanol/water (50/25/20, v/v/v) as the mobile phase. The detection reagent was composed of 1 g of diphenylamine, 1 g of aniline dissolved in 50 mL of acetone, and 5 mL of 85% H_3PO_4. Detection was performed by dipping the plate for 10 s in the reagent, followed by heating in an oven at 110 °C for 10 min.

Sucrose and Maltose Analysis. A standard GC procedure as described in the Pierce catalog (Pierce Chemical Co., 1988) was used for these analyses. Best separation was obtained with a 30-m DB-17 fused silica megabore column, 1.0-μm film thickness (J&W Scientific, Inc., Folsom, CA). The carrier and detector makeup gases were helium at 2 and 20 mL/min, respectively. The column oven temperature program was 165–185 °C at 1 °C/min, 185–260 °C at 5 C/min, and held at 260 °C for 10 min.

Materials. *Experimental Products. Raw Materials.* Unless otherwise stated, the coffee used was Santos NY 4-5 light-roasted with roasting loss of ca. 14%. Dried coffee husks, "the external envelopes of the dried coffee fruit" (ISO, 1984), a byproduct from both arabica and robusta dry-processed coffee, were used either as such, or after pelletization, or after pelletization and a thermal treatment corresponding to coroasting with coffee beans. Dried parchment, "the endocarp of the coffee fruit" (ISO, 1984), a byproduct from wet-processed arabica coffee, was used as such.

Autoclave Extraction. A stainless steel autoclave with a volume of 2 L, equipped with a magnetic stirrer, with double-wall heating and steam injection and with a sampling system, was used (Model BEP 280 Type III, Büchi, Uster, Switzerland). Before being extracted at high temperature (hydrolysis step), each sample for the autoclave trials was extracted (first stage) for 30 min at 100 °C to stimulate the extraction of soluble material that takes place in the low-temperature percolators of an industrial extraction train. These partially extracted samples were then extracted (second stage) in the autoclave at temperatures and for times representative of the full range of industrial practice.

Industrial Extraction. The conditions applied in the plant were as described in the Guggenheim and Stinchfield patent (1959).

Commercial Samples Analyzed. The 122 samples reported here were commercial soluble coffee powders from Australia, Brazil, Canada, Ecuador, Germany, Hungary, Japan, Mexico, Switzerland, United Kingdom, and the United States.

RESULTS AND DISCUSSION

Experimental Products. Pilot-plant and industrial-scale extractions were performed on either coffee beans of established quality or coffee beans with added known amounts of husks or other substances. The resulting experimental products were used to establish reference profiles of pure and adulterated soluble coffees.

Extraction Trials with Pure Roasted Coffee. The typical carbohydrate profile of pure soluble coffees was established on industrial countercurrent extracts of pure arabica or pure robusta beans. The carbohydrate compositions of 100% arabica, of 100% robusta, and of blended soluble coffees are given in Table I as the percentage of dry soluble solids (dmb = dry material basis).

These experiments were completed by a series of batch autoclave trials at the pilot-plant level, where all possible industrial extraction conditions were reproduced with arabica coffee: In each of these trials, the first stage of extraction, simulating extraction in the "cold" cells, was performed at 100 °C for 30 min. The duration and temperature of the second stage of extraction, corresponding to the hydrolysis step in the "hot" cells, varied between 30 and 240 min, at temperatures between 150 and 190 °C, respectively.

The extracts from the two stages were then combined before analysis. The carbohydrate composition of extracts thus obtained is given in Table II as the percentage of dry matter in the total extract. The variations in the free and total sugar profiles in Table II reflect range limits well beyond those normally found for pure coffees extracted under standard industrial production conditions: In the experimental samples, the higher levels of free glucose and fructose are always preceded by even greater increased levels of free galactose and mannose. In this case, free fructose and glucose are formed from mannose and galactose subunits released from polysaccharides. The most drastic conditions reported in Table II result in an almost total destruction of carbohydrate material. These results are consistent with those described by Kroplien (1974).

However, even under extreme conditions, the differences in profiles are far from those found with adulterated coffees as reported in later tables. For xylose, where no

Table II. Free and Total Carbohydrate Contents of Autoclave Extracts Made from Arabica (Santos NY 4-5) Roasted Coffee (% Dmb)

conditions of 2nd-stage extractn		free carbohydrate					total carbohydrate				
min	°C	Ara	Fru	Man	Glu	Gal	Xyl	Ara	Man	Glu	Gal
30	150	1.35	0.11	0.05	0.09	0.10	0.14	6.29	10.66	1.86	12.76
	160	1.74	0.10	0.08	0.10	0.19	0.16	6.00	8.95	1.40	18.40
	170	1.89	0.11	0.17	0.09	0.38	0.21	5.34	9.93	1.32	20.60
	180	1.44	0.17	0.43	0.10	0.79	0.17	3.55	13.09	1.29	18.00
	190	0.52	0.47	1.42	0.19	1.61	0.21	2.53	19.50	1.73	14.63
120	160	2.17	0.11	0.32	0.11	0.82	0.17	4.26	10.23	1.29	20.93
	170	1.19	0.24	0.82	0.14	1.55	0.20	2.65	13.40	1.45	16.40
	180	0.64	0.77	2.54	0.36	2.36	0.13	1.03	15.50	1.87	10.08
240	160	1.44	0.24	0.84	0.16	1.79	0.18	2.70	11.30	1.36	17.00
	170	0.62	0.57	2.02	0.27	2.31	0.14	2.01	13.70	1.86	11.48
	180	0.03	1.08	2.82	0.70	1.44	0.06	1.72	9.36	2.00	4.61
	190	0.00	0.03	0.43	0.01	0.01	0.08	2.05	5.91	1.66	3.19

Table III. Free and Total Carbohydrate Contents of an Autoclave Extract Made from a Substandard Arabica Coffee Containing 1% Residual Husks (% Dmb)

conditions of 2nd-stage extractn	
min	120
°C	170
free carbohydrate	
Ara	1.45
Fru	0.13
Man	0.48
Glu	0.14
Gal	0.88
total carbohydrate	
Xyl	0.23
Ara	3.55
Man	11.40
Glu	1.19
Gal	18.80

literature data are available, an estimate can be made based on the recent study of Bradbury and Halliday (1987), who found a maximum of 0.2% dmb total xylose in the green bean; if this sugar is thermally stable on roasting and is totally extracted, a maximum of approximately 0.3–0.5% dmb would be observed in the soluble coffee. Our experiments indicate that, at most, about 50% of the amount present in the green bean is recovered.

One trial, using dry-processed arabica coffee, containing 1% husks before roasting, showed a similar profile to pure coffee, as can be seen in Table III.

Extraction Trials with Pure Husks and with Pure Parchment. Similar weights of naked beans and husks are obtained in the dry processing of coffee. Dry coffee husks are light and bulky and are often pelletized by extrusion before being used as a low-cost fuel. The carbohydrate profiles of two-stage autoclave extracts from pelletized husks as such or after further thermal treatment corresponding to roasting with coffee beans are given in Table IV as the percentage of dry matter of the total extract (first stage, 30 min at 100 °C; second stage, 120 min at 170 °C).

The relatively high level of total xylose present, typical of woody material, is approximately the same for both pelletized and thermally treated husks, while levels of free glucose and fructose, the other important components of the husks, are lowered as a function of the thermal treatment. Xylose accounts for up to half of the extractable matter of parchment obtained from wet processing of coffee and is therefore free of adhering pulp. One exhaustive extraction gave 47.8% total xylose.

Trials Made by Coextraction of Roasted Coffee and of Husks. Industrial countercurrent extraction trials were conducted with mixtures of roasted arabica coffee, along with dry husks, pelletized husks, or thermally treated husks in different proportions. The resulting carbohydrate compositions are given in Table V as the percentage of dry matter of the extract: All samples exhibit a relatively high level of total xylose, which is essentially independent of the thermal treatment of the husks, while the levels of total and free glucose and of fructose are seen to be very sensitive to thermal treatment of the husks. For the darker thermal treatments, the carbohydrate profile of the soluble product differs from that of a pure soluble coffee only in the higher xylose content, which is not influenced by roasting.

These data have been completed with autoclave extraction trials on a mixture of 70% roasted Santos NY 4-5 and 30% untreated husks (w/w). Here again, the first stage of extraction has been standardized at 30 min at 100 °C and the carbohydrate composition of the combined extracts after various second-stage configurations is given in Table VI. A comparison with the results obtained with pure coffee (Table II) shows that for the same extraction conditions far higher levels of total xylose are always present.

Total and free glucose and fructose are also significantly higher, while the other carbohydrates are essentially unchanged.

Commercial Samples. The free and total carbohydrate contents of the 122 samples analyzed, all sold as pure soluble coffee, are given in Table VII; the samples have been arbitrarily subdivided into classes, on the basis of analytical data. In addition to this table, Figure 1 shows some typical HPLC profiles for the reference carbohydrates and for class A and B_1 products.

To complete the sugar profiles presented in Table VII, sucrose and maltose were also determined by a modification of the Pierce GC method. The results are given in Table VIII.

Table IV. Free and Total Carbohydrate Contents of Autoclave Extracts of Pelletized Husks (% Dmb)

	free carbohydrate					total carbohydrate				
thermal treatment	Ara	Fru	Man	Glu	Gal	Xyl	Ara	Man	Glu	Gal
none	2.67	18.93	0.90	15.41	0.66	5.00	3.71	1.18	15.73	2.69
light (200 °C, 13 min)	1.67	5.76	0.66	4.55	0.57	5.76	4.14	1.32	5.86	2.81
medium (209 °C, 13 min)	1.46	5.74	0.62	1.98	0.66	5.74	4.27	1.48	4.32	1.51

Table V. Free and Total Carbohydrate Contents of Industrial-Scale Products from Mixtures of Coffee and of Husks (% Dmb)

coffee/husks ratio (w/w)	type of husks	free carbohydrate					total carbohydrate				
		Ara	Fru	Man	Glu	Gal	Xyl	Ara	Man	Glu	Gal
85/15	nonpelletized	1.48	5.14	0.50	4.22	0.73	0.79	3.87	12.30	6.23	16.30
75/25	pelletized	1.39	4.60	0.63	3.00	0.74	1.55	4.14	11.40	4.51	14.60
85/15	thermally treated	2.16	0.25	0.26	0.21	0.51	0.60	5.55	9.60	1.59	19.00

Table VI. Free and Total Carbohydrate Contents of Autoclave Extracts from Mixtures of Arabica Roasted Coffee and Unpelletized Husks (% Dmb)

conditions of 2nd-stage extractn		free carbohydrate					total carbohydrate				
min	°C	Ara	Fru	Man	Glu	Gal	Xyl	Ara	Man	Glu	Gal
30	135	0.48	12.47	0.23	11.58	0.10	0.20	3.73	3.99	13.55	3.95
	150	0.95	10.47	0.22	9.46	0.17	0.32	4.05	5.20	11.20	8.84
	165	1.86	9.00	0.31	8.52	0.37	0.73	5.07	6.37	10.02	13.50
	180	1.73	8.29	0.58	7.74	0.60	1.13	3.44	9.64	9.30	12.06
	195	0.05	8.33	2.64	7.13	1.80	0.68	1.72	14.46	9.13	7.52
120	160	2.30	10.14	0.57	9.30	0.70	1.21	5.39	8.77	10.97	17.73
	170	0.95	9.86	0.88	8.63	1.36	1.24	2.85	12.73	10.33	14.00
	180	0.05	10.50	3.83	8.77	2.98	0.49	1.74	10.92	9.98	7.14
	190	0.05	11.40	2.18	9.82	0.83	0.18	1.72	6.16	11.08	3.34
240	160	1.93	10.20	0.97	9.24	1.43	1.38	3.49	9.97	10.40	15.10
	170	0.35	10.40	2.19	8.94	2.26	0.66	1.79	10.94	10.08	8.92

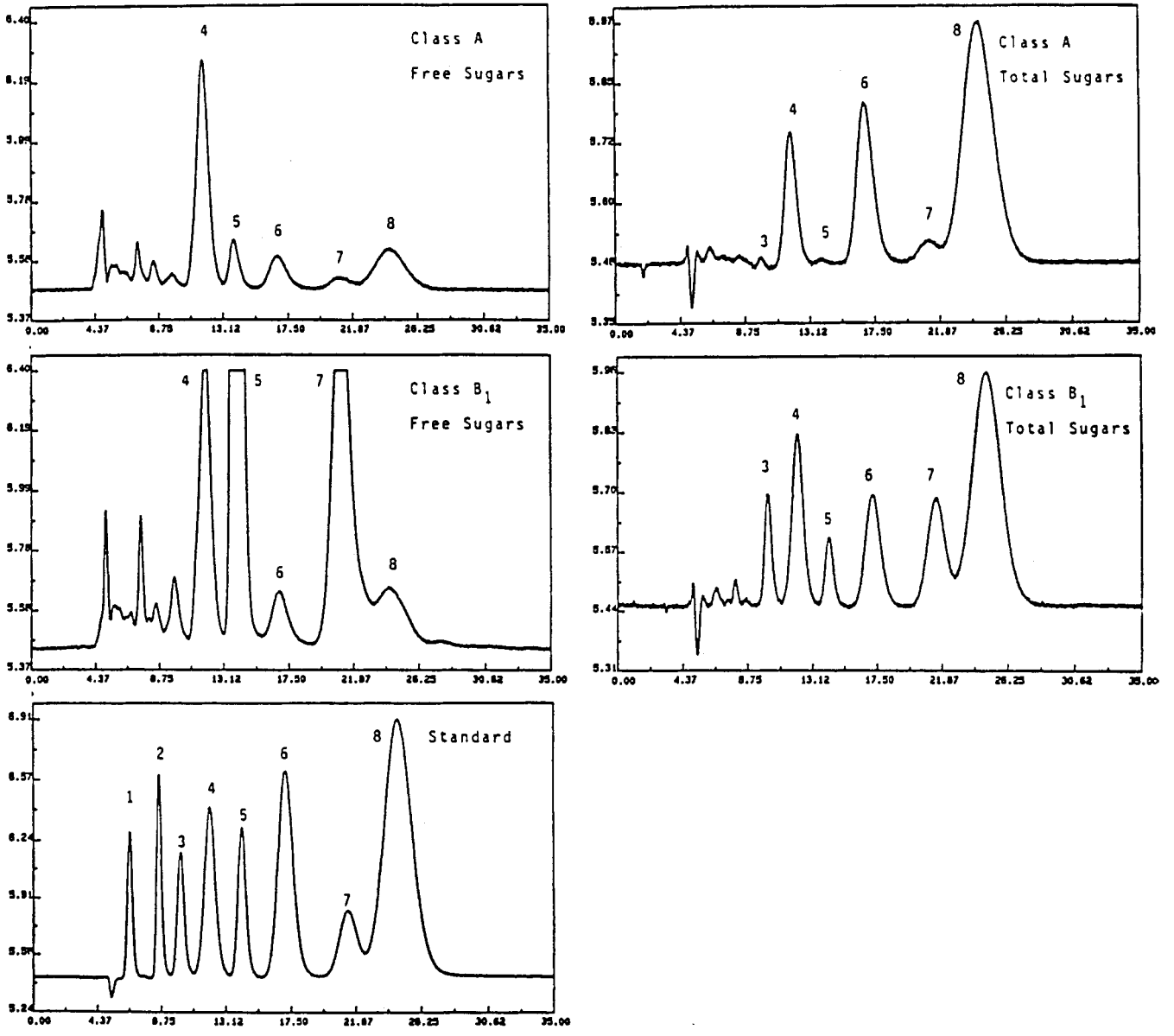

Figure 1. HPLC carbohydrate profile: 1, rhamnose; 2, ribose; 3, xylose; 4, arabinose; 5, fructose; 6, mannose; 7, glucose; 8, galactose.

Table VII. Free and Total Carbohydrate Contents of Commercial Products Sold as Pure Soluble Coffee (% Dmb)

class	no. of samples		free carbohydrate					total carbohydrate				
			Ara	Fru	Man	Glu	Gal	Xyl	Ara	Man	Glu	Gal
A	43		0.75–2.01	0.05–0.42	0.13–2.62	0.00–0.43	0.27–0.72	0.11–0.28	2.35–5.80	10.2–19.7	0.57–1.51	13.5–24.7
		av	1.17	0.12	0.53	0.12	0.47	0.17	3.98	14.2	0.97	19.5
B_1	42		0.59–1.61	1.17–5.13	0.25–0.77	0.60–3.08	0.29–0.90	0.45–1.54	3.39–5.28	5.10–10.4	1.80–5.77	12.0–22.2
		av	1.11	2.91	0.51	1.91	0.57	0.97	4.19	7.50	3.78	16.2
B_2	23		0.78–1.48	0.15–0.74	0.12–0.57	0.08–0.51	0.14–0.68	0.56–1.95	3.53–5.21	4.80–10.5	1.20–3.03	14.0–26.5
		av	1.07	0.37	0.32	0.26	0.49	1.01	4.19	7.10	1.88	18.8
C	14		0.33–1.21	0.35–4.14	0.19–0.57	0.94–3.67	0.20–0.58	0.15–1.20	1.96–4.09	3.90–6.90	10.7–50.8	9.00–16.9
		av	0.92	2.59	0.45	2.38	0.44	0.66	3.17	5.80	24.6	12.8

Table VIII. Sucrose and Maltose Contents of Commercial Products Sold as Pure Soluble Coffee (% Dmb)

class	no. of samples		sucrose	maltose
A	13		0.06–0.30	0
		av	0.17	
B_1	23		0.25–1.27	0
		av	0.54	
B_2	20		0.00–1.05	0
		av	0.34	
C	12		0.24–3.00	0.71–2.21
		av	1.22	1.43

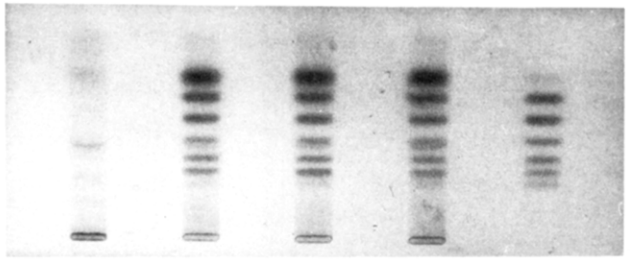

Figure 2. (1) Class A soluble coffee; (2–4) class C products; (5) maltooligosaccharides, polymerization degree 2–7.

The pure soluble coffee profile is characterized by a low free-sugar level, except for arabinose, as expected. Quantities of free glucose and fructose are at most 0.4% and ca. 0.6%, respectively. With respect to the total sugar profile, the major observation is the consistently low level of xylose.

Class A contains commercial products having the carbohydrate profile of pure soluble coffee, as shown by the good agreement with values given in Tables I and III.

Class B contains samples characterized by a high level of total xylose, corresponding to the coextraction of coffee with up to 25% coffee husks and/or parchment. This class can be further subdivided into two classes based on glucose and fructose levels, which are high [B_1] or normal [B_2] according to whether pelletized husks or roasted husks/parchment have been coextracted with roast coffee. The similarity of sugar profiles of the products classified B_1 and those of the experimental products made from mixtures of pelletized coffee husks and roasted coffee is excellent. In class B_2, the sugar profiles of the products result from the presence either of roasted pelletized coffee husks or of parchment. The presence of mannitol (Davis et al., 1989) and inositol (Zuluaga Vasco, 1980) in the pulp can distinguish between these two types of adulteration.

Class C samples are all characterized by high levels of total glucose. The presence of maltose as shown in Table VIII is indicative of the incorporation of maltodextrins. This was confirmed by TLC analysis of intact sugars (Figure 2). Some samples contained enhanced levels of sucrose, probably indicating an addition of (caramelized) sugar.

The lower levels of total mannose encountered with samples classified B and C may be explained by the fact that mannan stability is very sensitive to pH during extraction.

Most of the soluble coffee on the world market is undoubtedly manufactured from authentic raw material, but evidence is provided here that a number of other products sold as pure soluble coffees contain undeclared additives. The sugar profile methodology presented here gives the possibility to detect these.

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Registry No. Ara, 147-81-9; Gal, 59-23-4; Man, 3458-28-4; Fru, 57-48-7; Glu, 50-99-7; Xyl, 58-86-6; sucrose, 57-50-1; maltose, 69-79-4; maltodextrin, 9050-36-6.

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