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Registry No. Lys, 56-87-1; Thr, 72-19-5.

Chromatographic Profile of Carbohydrates in Commercial Coffees. 2. Identification of Mannitol

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Mannitol, a polyhydric sugar alcohol, has been identified for the first time in coffee products. Its presence in some commercial soluble coffees at levels above 0.30% indicates adulteration.

Recent studies of the carbohydrate composition of soluble coffee powders (Blanc et al., 1989) have provided evidence that coffee husk extracts are present in some commercial soluble coffees at concentrations as high as 25%. At these levels the quantities of undeclared materials in the product after industrial processing cannot be considered a nondeliberate "defect" in the product (Jobin, 1982).

Inositol is the only polyhydric alcohol reported earlier in roasted coffee beans and instant coffee powder (Mishkin et al., 1970). Zuluaga Vasco and Tabacchi (1980) found 1.2% inositol in the carbohydrate fraction of fresh wet processed coffee pulp.

Mannitol, also a polyhydric alcohol, is frequently found in exudates of plants such as the flowering ash, olive, and plane trees and in marine algae in concentrations in excess of 20% (Lohmar, 1974). It has now been identified in the carbohydrate fraction of pelletized coffee husks and also in certain commercial soluble coffees, where its presence confirms adulteration: its behavior in the conditions of soluble coffee manufacture will be discussed here.

EXPERIMENTAL PROCEDURES

Standards and Reagents. Pure sugars and polyhydric alcohol standards were obtained from local supply houses. STOX, oxime internal standard reagent, and *N*-(trimethylsilyl)imidazole were obtained from Pierce Chemical Co. (Rockford, IL). ABH, the postcolumn derivatization agent, was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Free Carbohydrate and Polyhydric Alcohol Analyses. *Apparatus.* A Pierce derivatization system, consisting of a Reacti-Therm heating module, a Reacti-Block, and 3-mL Reacti-Vials, was used for preparation of volatile derivatives. Samples were analyzed by GC on a Vista 6000 instrument (Varian, Palo Alto, CA) equipped with a 30-m DB-17 fused silica megabore column, 1.0-μm film thickness (J&W Scientific, Folsom, CA). Helium was used as the carrier gas (2 mL/min) and detector make-up gas (20 mL/min). The column oven temperature program was 165–185 °C at 1 °C/min, 185–260 °C at 5 °C/min, and 260 °C isothermal for 10 min. GC peaks were integrated

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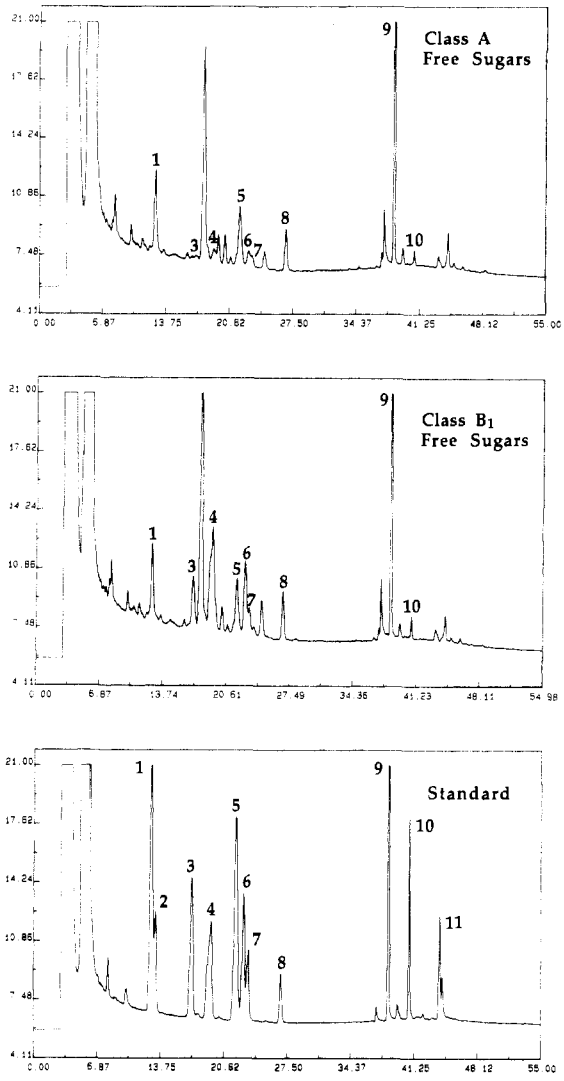

Figure 1. GC carbohydrate profiles: 1, arabinose; 2, xylose; 3, mannitol; 4, fructose; 5, mannose + galactose; 6, glucose; 7, glucose + galactose; 8, inositol; 9, internal standard; 10, sucrose; 11, maltose.

with an HP-1000 (Hewlett-Packard Co., Avondale, PA) data acquisition system.

A 4000 series quadrupole gas chromatograph–mass spectrometer (GC-MS) equipped with an INCOS data treatment system (Finnigan-MAT, Palo Alto, CA) was used for identification. The fused silica GC capillary column was coupled directly to the MS. EI mass spectra were recorded at 70 eV, at an ion source temperature of 180 °C, and at a scan rate of 2 scans/s over a mass range m/z 45–650.

Analytical Procedure. Soluble coffee (100 mg) was treated according to method 16 in the Pierce catalog (Pierce, 1989), and 0.5 μL of the volatile derivative solution was injected into the GC.

Total Carbohydrate Analysis. *Apparatus.* A Varian Model 5060 HPLC with a UV-10 variable-wavelength detector was combined with a Model PCRS 520 (Kratos, Ramsay, NJ) postcolumn derivatization system and an HP-1000 (Hewlett-Packard) data acquisition system. Separations were performed by using a CHO-682 column (Interactions Chemicals, Mountain View, CA) protected by a Micro-Guard Cation-H precolumn (Bio-Rad, Richmond, CA).

Analytical Procedure. Soluble coffee (200 mg) was hydrolyzed by refluxing 2 h in 10 mL of 0.6 N HCl. These mild conditions have been found sufficient for the hydrolysis of soluble coffee carbohydrates.

After cooling, the solution, diluted to 100 mL with distilled water, was filtered through an HA 0.45-μm filter (Millipore, Bedford, MA) and cleaned on a C-18 Sep-Pack cartridge (Waters Associates, Milford, MA). Twenty microliters of the final solution was separated by HPLC using distilled water at 0.4 mL/min as the mobile phase. The eluted components were detected at 400 nm by using ABH as the postcolumn derivatization agent (Femia and Weinberger, 1987).

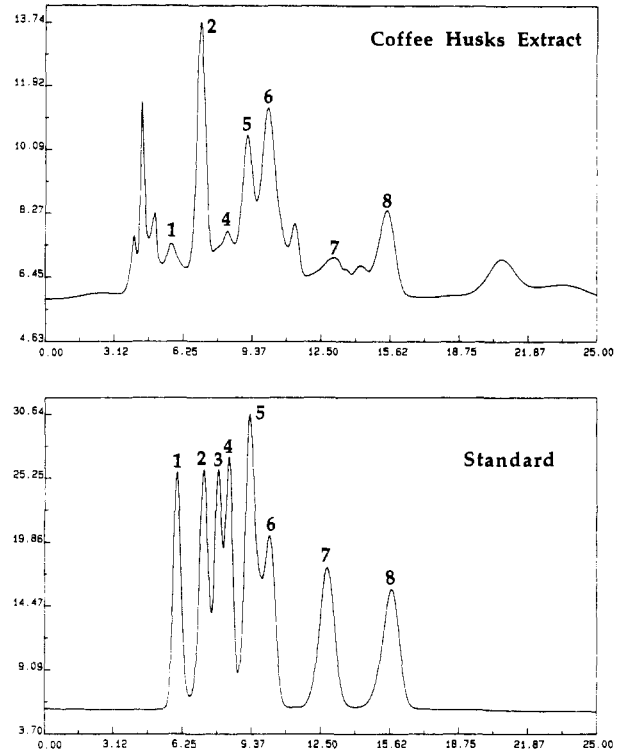

Figure 2. HPLC carbohydrate profiles: 1, sucrose; 2, glucose; 3, xylose; 4, galactose; 5, arabinose + mannose; 6, fructose; 7, inositol; 8, mannitol.

Samples. *Raw Materials.* The coffees used were Santos NY 2/3-8 and substandard arabica beans, light roasted to a roasting loss of ca. 14%. Dried coffee husks from arabica dry-processed coffee were either used as such or after pelletization or after pelletization and a thermal treatment corresponding to coroasting with coffee beans.

Experimental Products. Reference samples were obtained by autoclave extraction in an indirectly heated 2-L stainless steel autoclave, Model 4642, equipped with a stirrer and a sampling system (Parr Instruments Co., Moline, IL). These samples were prepared to determine the mannitol content of powders made from roast coffee and either raw or roast husks. As starting material, either coffee beans of acceptable commercial quality or coffee beans with known amounts of husks added were used.

Commercial Samples. One hundred forty-five soluble coffees from Brazil, Canada, Colombia, Ecuador, El Salvador, Germany, Japan, Mexico, Paraguay, Switzerland, the United Kingdom, and the United States were analyzed.

Identification of Polyhydric Alcohols. In this study, the identity of both mannitol and inositol has been established by GC retention time comparison with authentic samples (elution times relative to the internal standard of 0.44 and 0.70, respectively), as shown in Figure 1; by HPLC [RI detection, after elution with distilled water from a μ-Spherogel carbohydrate column (Beckman Instruments, San Ramon, CA)], as shown in Figure 2; and by GC–MS electron impact mass spectra, as shown in Figure 3.

RESULTS AND DISCUSSION

Levels of inositol between 0.21% and 0.31% have been found in coffee husks and between 0.20% and 1.03%, average 0.50%, in roasted coffee extract.

Mannitol has now been found for the first time in a coffee material: it is present in dried coffee husks in amounts of 1.61–2.03%.

Very low concentrations of mannitol up to 0.05% have been detected in commercially sound green coffee. Extract

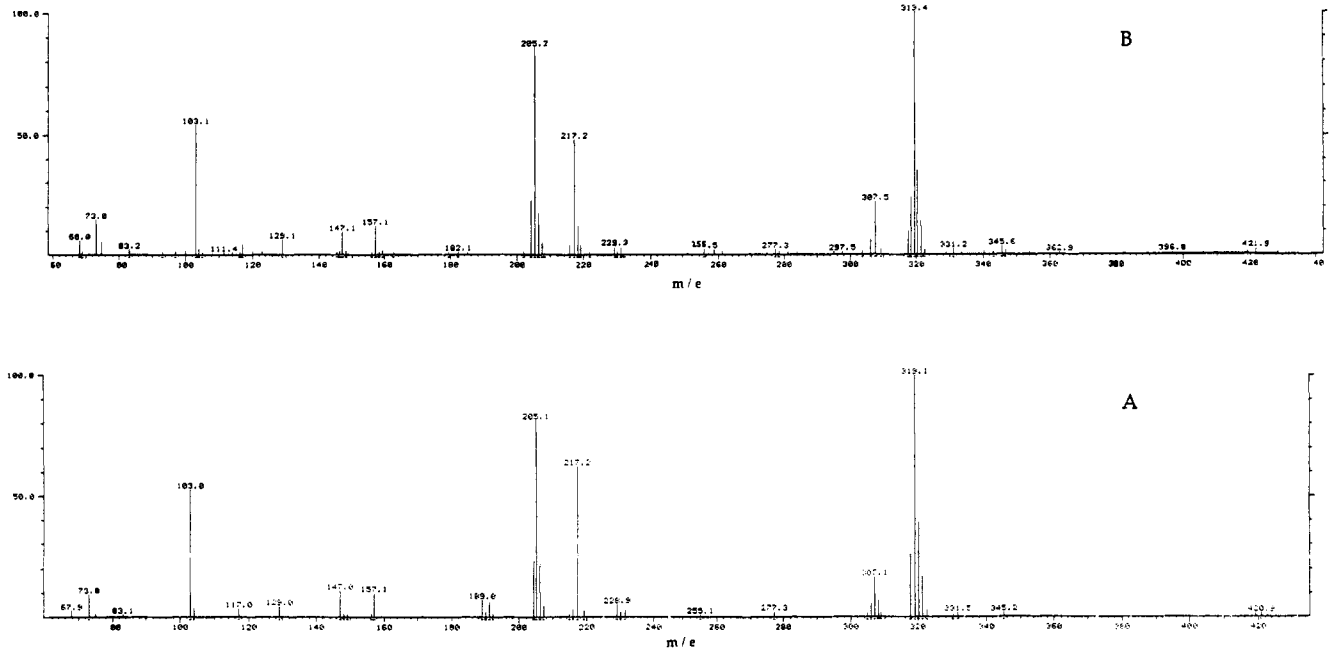

Figure 3. EI mass spectra of the per-*o*-trimethylsilyl derivatives of the pure mannitol standard (lower) and a compound isolated from coffee husk extract (upper).

Table I. Stability of Mannitol during Roasting (Percent Mannitol Recovered by Water Extraction[a])

thermal treatment	% recovered[b]
untreated	95.1
light roasted (200 °C, 13 min)	94.6
medium roasted (209 °C, 13 min)	103.4

[a] Double-stage extraction: 30 min at 100 °C and 2 h at 170 °C; water/husk ratio 20:1. [b] Relative to 2.03% mannitol found by direct analysis of untreated husks.

Table II. Stability of Mannitol during Extraction (Percent Mannitol Recovered by Water Extraction[a])

temp, °C	% recovered[b]
160	85.7
170	76.4
180	74.5

[a] Single stage extraction for 2 h; water/husk ratio 20:1. [b] Relative to 1.61% mannitol found by direct analysis of untreated husks.

made in the pilot plant at 51.09% yield from roasted Colombian beans (12 defects/500 g) contained 0.09% mannitol, and extract made at 51.80% yield from roasted Santos beans (26 defects/300 g) contained 0.08% mannitol. Concentrations of mannitol of the same order (average, 0.09%, range, 0.02–0.22%) have been detected in industrial extracts made from commercially sound beans. Such traces of mannitol present correspond to the very small amounts of husk material left on the beans after the industrial cleaning process (precisely defined small levels of husks, counted among the defects, are tolerated in commercial coffee as nondeliberate).

The presence of much larger amounts of mannitol has also been established in a few products commercialized as pure soluble coffee powders; this information provides a further indication of the undeclared use of husks in some commercial products.

Stability of Mannitol. The percentage of mannitol recovered after different thermal treatments is given in Table I. The stability of mannitol during high-temperature extraction is shown in Table II.

Husks of different mannitol content were used to establish the recovery values reported in Tables I and II. Independent of husk origin, a recovery of 95% was obtained: this indicates that neither single-stage extraction nor the double-stage extraction normally used in soluble coffee plants degrades mannitol.

Commercial Samples Analyzed. The carbohydrate profile of 145 samples of commercially available soluble coffee powders, sold as pure coffee in 12 producing or consuming countries, have been determined and grouped

Table III. Carbohydrate Content of Commercial Soluble Coffees (% dmb)

		sugar alcohols		free carbohydrates				total carbohydrates	
class	no. of samples	mannitol	inositol	fructose	glucose	sucrose	maltose	xylose	glucose
A	56	0.02–0.22	0.20–1.03	0.09–0.52	0.08–0.54	0.01–0.64	0	0.00–0.32	0.61–1.74
av		0.09	0.50	0.29	0.30	0.20		0.20	1.15
B_1	42	0.25–1.85	0.22–0.99	1.12–5.19	0.53–3.29	0.05–1.43	0	0.41–3.15	1.65–5.71
av		0.65	0.49	2.38	1.43	0.45		1.07	3.01
B_2	6	0.12–0.88	0.31–1.09	0.45–1.09	0.40–0.92	0.18–1.01	0	0.56–2.00	1.62–2.52
av		0.33	0.55	0.68	0.69	0.33		1.05	1.94
B_3	8	0.32–0.87	0.31–0.76	0.27–0.63	0.17–0.74	0.00–0.26	0	0.25–0.35	1.19–1.50
av		0.46	0.57	0.39	0.33	0.05		0.30	1.36
B_4	12	0.10–0.32	0.33–0.74	0.15–0.71	0.20–0.68	0.01–0.36	0	0.46–1.69	0.90–2.21
av		0.20	0.49	0.39	0.37	0.24		0.83	1.46
C	21	0.10–1.18	0.20–0.60	0.46–4.47	0.33–3.64	0.19–5.59	0.00–3.30	0.01–3.24	3.69–38.10
av		0.48	0.34	2.53	1.87	1.39	1.52	0.80	15.86

into classes according to the type of adulterant (Blanc et al., 1989), as given in Table III.

Classification of samples into groups A (pure coffee) and C (containing added maltodextrin or caramelized sugar) is unaffected by determination of mannitol, but group B products could be divided further when this sugar alcohol was included in the evaluation.

Subgroup B_1 contains commercial samples prepared from mixtures of coffee with untreated or pelletized husks and is characterized by high levels of mannitol, free fructose and glucose, and total xylose.

Subgroup B_2 contains products prepared from mixtures of coffee and thermally treated husks and is characterized by elevated quantities of mannitol and total xylose, but with nearly normal levels of free fructose and glucose.

A few samples, classified as B_3, were found in which mannitol was present in amounts typical of group B_2 but in which the total xylose was only slightly elevated.

Finally, some samples, classified as subgroup B_4, are identified by their high total xylose content, while mannitol and the remainder of the sugars are present in near normal amounts.

The relative content of mannitol and xylose in the products classified B_3 and B_4 could be explained by extraction either of mixtures of coffee and husks under special conditions or of mixtures of coffee with byproducts of wet processed coffee, such as parchment.

The majority of soluble coffee powders sold in the world market are made from good quality coffees, but a mannitol content exceeding 0.3% in a sample constitutes further evidence that the product contains undeclared material.

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Registry No. Mannitol, 69-65-8; inositol, 87-89-8; fructose, 57-48-7; glucose, 50-99-7; sucrose, 57-50-1; maltose, 69-79-4; xylose, 58-86-6; galactose, 59-23-4; arabinose, 147-81-9; mannose, 3458-28-4.