Monosaccharides in Roasted and Instant Coffees

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The monosaccharides of roasted and instant coffees were analyzed. A one-step procedure for quantitative isolation of monosaccharides from coffees was developed. The sugars were quantified by thin-layer densitometry of their colored reaction products with 4-aminobenzoic acid. Roasted coffee extracts contained glucose (0–0.9%), fructose (0–0.9%), arabinose (0–0.1%), and sometimes a trace of galactose. Contents were low in dark and high in light roasts. Instant coffees contained arabinose (0.4–2.5%), galactose (0.2–0.9%), mannose (0.1–1.0%), glucose (0–0.3%), fructose (0–0.5%), and traces of ribose and xylose. Lab-scale extraction of roasted coffees at temperatures up to 200° proved that arabinose, galactose, and mannose were formed by hydrolysis of polysaccharides. The relative composition of the monose fraction is likely to reflect the hydrolysis conditions in the manufacture of instant coffees.

For the production of "instant coffees" (instantly soluble coffee powders) the commercial coffee extraction is carried out at temperatures well above 100° (Sivetz and Foote, 1963). Under household conditions, on the other hand, coffee is extracted with boiling water at atmospheric pressure.

Paper chromatography (Streuli, 1970; Thaler, 1957) indicates differences between the monosaccharide patterns of roasted coffee beans and of instant coffees. Free arabinose and galactose could be typical constituents of the latter (Streuli, 1970).

It was our objective to develop a quick and reliable method for the quantitative determination of the monosaccharides as a basis for differentiation between both types of coffee extract. As a first step, the monosaccharides of instant coffee were unambiguously identified. Subsequently a procedure was elaborated for the separation of the monose fraction from all other coffee components, followed by thin-layer chromatography and densitometric quantification of the individual sugars. Additionally, various coffees were extracted in a lab-scale autoclave for different lengths of time and at different temperatures. The compositions of the monose fractions were compared with the commercial products.

EXPERIMENTAL SECTION

Coffees. Roasted coffees used for autoclave extractions were experimental blends supplied by Ibenco, Wedel, W. Germany. The correlation between degree of roast and glucose and fructose contents of coffee was investigated on two experimental blends supplied by The Coca-Cola Co., Foods Division, Houston, Tex., USA. All other roasted and instant coffee samples were of commercial origin, manufactured either in the United States or in West Germany.

Thin-Layer Chromatography. *Plates.* Precoated silica plates were used, with or without fluorescence indicator (E. Merck, Darmstadt, Germany).

Sample Application. Sample solutions were applied to tlc plates with a repeating dispenser PB 600-1 with a 25-μl syringe (Hamilton, Den Haag, the Netherlands).

Solvent Systems. Ethyl acetate–acetone–water, 40:50:10 (v/v), five ascents (Bancher *et al.*, 1964), was used for qualitative chromatography of free sugars. Plates for sugar quantification were developed with ethyl acetate–acetone–water, 40:53:7 (v/v), five ascents. Sugar osazones were separated with 2-butanone–benzene–methanol–saturated aqueous borax (20°), 43.5:43.5:11:2 (v/v) (modified from Haeseler and Misselhorn, 1966).

Spray Reagents. Diphenylamine–aniline–phosphoric acid (Bancher *et al.*, 1964) and dimedone (for ketoses only) (Adachi, 1964) served for detection of monoses on thin-layer plates. 4-Aminobenzoic acid–phosphoric acid was used for sugar quantification. The reagent is prepared as follows. *p*-Aminobenzoic acid (300 mg) is dissolved in glacial acetic acid (44 ml), followed by the addition of water (3 ml) and 85% phosphoric acid (3 ml). Plates are sprayed evenly (*ca.* 15 ml) and heated to 100° for 20 min.

Autoclave. A lab-scale stainless steel autoclave with a volume of 200 ml was used. The apparatus was equipped with electrical heating, magnetic stirring, thermocouple, and automatic temperature control (C. Roth, Karlsruhe, Germany, no. 20-80.64).

Autoclave Treatment of Coffee. *One-Step Extraction.* The autoclave was filled with double-distilled water (150 ml). Roasted coffee (10 g, ground to a particle size of 0.2–0.5 mm) was slowly poured into the magnetically stirred water. The autoclave was closed and heated to the desired temperature. Stirring was continued throughout the whole experiment. The time necessary to reach the final temperature varied between 12 min at 120° and 21 min at 200°.

After 180 min of heating, (including the warming-up period), the autoclave was rapidly cooled down in tap water. Within 1 min the contents reached a temperature of 66 ± 12°, low enough to prevent further reactions.

The reaction solutions were transferred quantitatively to an Erlenmeyer flask which was stoppered and stored overnight in a refrigerator. Subsequently the cooled solutions were centrifuged for 15 min at 3000 rpm; 50 ml of the supernatant was used for determination of soluble solids and the rest was freeze-dried.

Discontinuous Extraction. The procedure followed largely the one used for one-step extraction, but differed in some important details. Roasted coffee (10 g, ground to a particle size of 0.2–0.5 mm) was extracted exhaustively on a coffee filter funnel with boiling, double-distilled water (five portions of 100 ml each). Two 25-ml portions of the extract served to determine soluble solids in duplicate. The wet coffee grounds were transferred with double-distilled water (150 ml) to the autoclave. The suspension was heated to 120° and stirred as described above.

After a total heating period of 60 min, the autoclave was rapidly cooled and opened. The solution was carefully decanted and the grounds were rinsed with water (*ca.* 50 ml). The volume of the combined extract and washings was determined prior to storing the solution in a refrigerator overnight. Fresh water (150 ml) was added to the coffee grounds in the autoclave and the extraction procedure was repeated at 140°. Subsequently, in the same manner, extraction temperatures of 160, 180, and 200° were applied. After the last extraction the coffee grounds were discarded. Further working-up steps of the extracts were the same as described in one-step extraction.

Coca-Cola GmbH, Essen, West Germany.

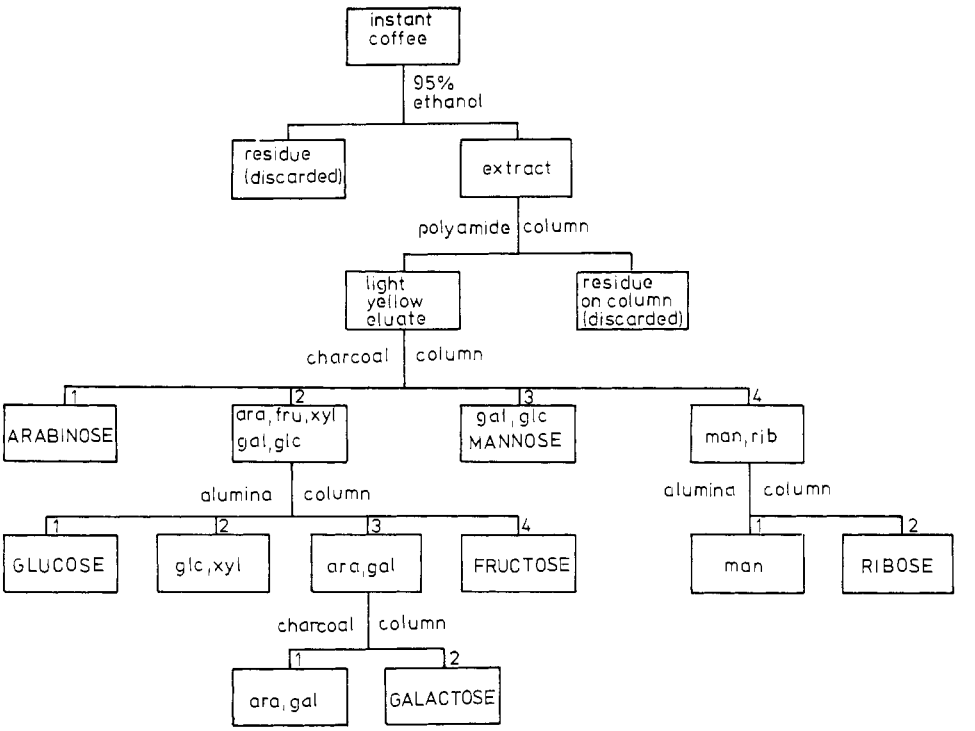

Figure 1. Isolation of instant coffee monosaccharides, flow diagram. ara, L-arabinose; fru, D-fructose; gal, D-galactose; glc, D-glucose; man, D-mnose; rib, D-ribose; xyl, D-xylose. Capital letters indicate isolation of a sugar from that fraction. For details, see Experimental Section.

Determination of the Total Solubilized Matter (Extract). Twenty-five-milliliter portions of each cooled and centrifuged extract were pipetted in duplicate to evaporation dishes. The solutions were evaporated and dried to constant weight in a drying oven at 105°.

Qualitative Analysis of Instant Coffee Monoses. *Column Chromatographic Methods.* It was observed that monosaccharide mixtures can be partially separated by charcoal in accordance with the desorption characteristics reported by Whistler and Durso (1950). The sugars are eluted in the order: arabinose, fructose, xylose, galactose, glucose, mannose, and ribose.

In previous work we found that aqueous solutions of monoses interact with alumina, the strength of interaction depending on the acidity of the alumina and on the structure of the sugars (Kröplien, 1973). The order of elution from neutral alumina columns is: glucose, xylose, arabinose and galactose, mannose, fructose, and ribose. The last two sugars are best desorbed with the aid of dilute acetic acid. With the exception of xylose, all the monosaccharides from instant coffee could be isolated by fractionation of one sample according to the following procedure.

Sugar Isolation (see Figure 1). Instant coffee (100 g) was shaken with 95% ethanol (2 l.) for 20 hr at room temperature. The extract was filtered and concentrated *in vacuo* to a thick syrup, and this was dissolved in water (150 ml). The solution was extracted with chloroform (150 ml), then passed through a polyamide column (50 × 5 cm) (Woelm, Eschwege, Germany) which was eluted with water. The yellow effluents (200 ml) were concentrated to about 20 ml. The concentrate was chromatographed in five portions on a charcoal column (60 × 2 cm) Merck, Darmstadt, Germany) with deionized water, elution being accelerated by means of compressed air. The effluents were pooled into four fractions according to composition, as revealed by thin-layer chromatography. The bulk of noncarbohydrate material (mainly salts) was removed by evaporation and extraction of the residue with ethanol.

From fraction 1 crystalline L-arabinose was obtained by evaporation and treatment of the syrupy residue with glacial acetic acid. Fraction 2 was rechromatographed on a neutral alumina column and yielded pure glucose, pure fructose, as well as a further fraction which, after chromatography on a charcoal column, afforded crystalline D-galactose. Fraction 3 yielded D-mannose as the crystalline phenylhydrazone. Fraction 4 was also chromatographed on an alumina column and pure ribose was isolated.

For the isolation of xylose a different separation path had to be followed. The effluent from the polyamide column was largely freed of arabinose by two subsequent separations over a charcoal column. Final chromatography on an alumina column yielded pure xylose.

Identification. L-Arabinose: mp and mixed mp 156° (dec) (lit. 160°, dec) (Weast, 1971); $[\alpha]^{20}D$ +102.3° (*c* 0.8, water), lit. +104.5° (*c* 3) (Weast, 1971). Calcd for $C_5H_{10}O_5$: C, 40.0; H, 6.67. Found: C, 39.23; H, 6.69.

D-Mannose phenylhydrazone: mp and mixed mp 199° (lit. 199–200°) (Hirst *et al.*, 1947). Calcd for $C_{12}H_{18}N_2O_5$: C, 53.33; H, 6.67; N, 10.37. Found: C, 52.95; H, 6.70; N, 10.19.

D-Galactose: $[\alpha]^{20}D$ +80.1° (*c* 0.8, water) (lit. + 80°) (Wolfrom *et al.*, 1962). Calcd for $C_6H_{12}O_6$: C, 40.0; H, 6.67. Found: C, 39.04; H, 6.74. Methylphenylhydrazone, mp and mixed mp 193° (lit. 189°) (Hirst *et al.*, 1947).

In view of the not entirely satisfactory elemental analysis, L-arabinose, D-galactose, D-galactose methylphenylhydrazone, and D-mannose phenylhydrazone were also identified by their ir spectra. These were indistinguishable from spectra of authentic compounds (UNICAM SP 200, KBr pellets).

The sugars glucose, fructose, xylose, and ribose were identified only by thin-layer chromatography. Free sugars were indistinguishable from authentic samples by chromatography and cochromatography. In addition, osazones were prepared of all the sugars and of corresponding authentic material. Tlc and cochromatography showed that the osazones of isolated glucose, mannose, and fructose were indistinguishable from authentic glucosazone. Likewise, osazones of isolated arabinose and ribose, of galactose, and of xylose were identical with arabinosazone, galactosazone, and xylosazone, respectively.

Quantitative Analysis of Coffee Monosaccharides.

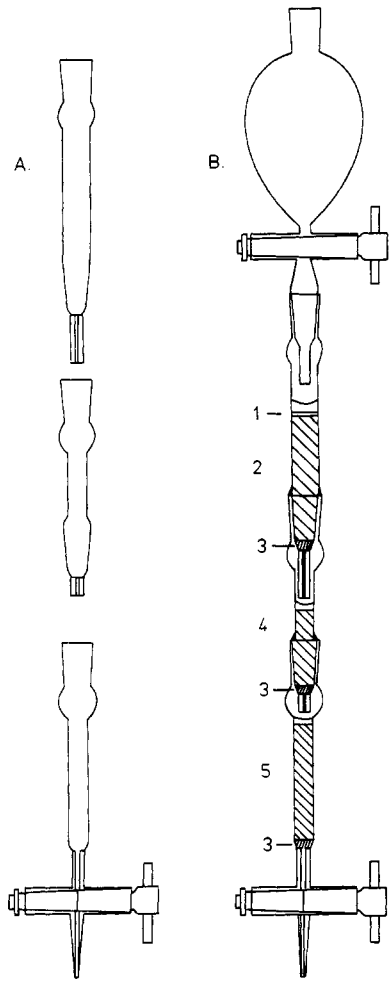

Figure 2. Combination column. A, spare parts; B, column ready for operation; 1, wire net; 2, charcoal + polyamide; 3, cotton wool plug; 4, cation exchanger; 5, anion exchanger. For details, see Experimental Section.

Sample Preparation: Roasted Coffee. Roasted and ground coffee (3.00 g) was added to hot redistilled water (24 ml) in a centrifuge tube and kept in a boiling water bath for 10 min with occasional stirring. After cooling, the sample was made up to its original weight with redistilled water and centrifuged, and 12 ml of the supernatant was lyophilized. Extraction yields varied between 19.1–28.5% and averaged 23.1% for all samples examined, based on coffee dry matter. A 10% solution (w/v) of the freeze-dried material in 1.5% ethanol (95% ethanol–redistilled water, 1.5:98.5) was subjected to chromatography.

Sample Preparation: Instant Coffee. Instant coffee (1.00 g) was dissolved in 10 ml of 1.5% ethanol and the centrifuged solution used for chromatography.

Sample Preparation: Autoclave Extracts. A 10% solution (w/v) of the freeze-dried extracts in 1.5% ethanol was prepared. If turbid, the solution was centrifuged prior to column chromatography.

Combination Column: Design and Operation. A combination column (Figure 2) consists of a sequence of three small columns filled with (top to bottom) charcoal + polyamide (0.5 + 2.0 g), cation exchanger (SE-Sephadex C-25, H^+, *ca.* 1.3 g), and anion exchanger (DEAE-Sephadex A-25, Ac^-, *ca.* 1.0 g). (Charcoal: Merck, Darmstadt, Germany; polyamide: Woelm, Eschwege, Germany; Sephadex: Pharmacia, Uppsala, Sweden).

Column Dimensions. Upper column: total length (t.l.) 150 mm, inner diameter (i.d.) 12.5 mm, with capillary outlet (i.d. 1 mm). Middle column: t.l. 115 mm, i.d. 10 mm, with capillary outlet (i.d. 1 mm). Lower column: t.l. 175 mm, i.d. 10 mm, with capillary outlet and Teflon stopcock. Approximate column bed heights: 65, 40, and 60 mm.

As a solvent reservoir, a dropping funnel (100 ml) with Teflon stopcock is used. All parts are linked together by standard ground glass joints (14.5 mm).

One milliliter of the coffee solutions prepared as described previously was applied to a combination column and eluted with 1.5% ethanol. Elution was monitored by thin-layer chromatography. A total of 11 ml (void volume) was discarded. The next 10 ml, collected in a volumetric flask, contained all the monosaccharides and were used for quantitative determination of the individual sugars.

Thin-Layer Densitometry of Monose Reaction Products with p-Aminobenzoic Acid. It was found that the very sensitive reaction of sugars with *p*-aminobenzoic acid (Leopold, 1962; Saini, 1966) gave stable colors on silica plates without background coloration, permitting densitometric quantitative analysis. Using a flying-spot densitometer TLD 100 (Vitatron, Dieren, the Netherlands) with an integrating recorder UR 400 (Vitatron) attached, monosaccharides could be quantified in the range of 0.01–2 μg/spot. The weight (W)-integrator response (I) curve was not completely rectilinear: $W_1/W_2 = (I_1/I_2)^{1.17}$. (The exponent was determined experimentally.) Relative absorbances of various monoses were: glucose and galactose, 100; fructose, 95; mannose, 87; arabinose, ribose, and xylose, 78. Densitometry was performed in transmission, with experimental conditions as follows: filter, 362 nm; mode, absorption minus; diaphragm, 0.5 mm; level, C; zero *c*, 6; damping, 2; span, *ca.* 8.50; eccentricity, 3 mm; linear velocity, 0.5 cm/min; recorder chart speed, 0.5 cm/min; integrator sensitivity, 7.

For determination of coffee monoses, constant volumes of the solution obtained from the combination column and of a suitable standard solution (arabinose, galactose, and mannose for instant coffee; glucose and fructose for roasted coffees) were spotted alternately five times each on a thin-layer plate (preferably 0.1–0.5 μg of a single sugar per spot). Subsequently, the amount of each sugar was calculated from the average integrator responses of standard and unknown according to the formula given above. At ≥0.1 μg/spot, the relative standard deviation is s = 2.8%.

Galactose, mannose, arabinose, and ribose are well separated by the glc system used and hence quantified without difficulty. Glucose will give a defined peak only if its concentration exceeds 10–15% of that of galactose; otherwise it will form a shoulder in the galactose peak. For separation of ribose and xylose a thin-layer plate has to be developed 7–8 times, with the running distance increased to 15 cm. Mannose and fructose are not separable in this system and, if present simultaneously, are determined together.

Thin-Layer Densitometry of the Fructose-Dimedone Reaction Product. The color reaction of ketohexoses with dimedone (Adachi, 1964) is less sensitive than the reaction with *p*-aminobenzoic acid but could be adapted to render the quantification of at least 0.1 μg of fructose/spot feasible. Under these modified conditions (heating of the sprayed thin-layer plate to 110° for 10 min), reactivity of aldose as compared to ketoses was still lower by *ca.* one order of magnitude. The method permitted thin-layer densitometric determination of fructose in the presence of up to a *ca.* 6–8-fold excess of mannose. A linear correlation was observed between 0.1 and 0.5 μg of fructose/spot. Two fructose standards (0.1 and 0.4 μg) and an adequate volume of the unknown with a fructose content within this range were applied alternately to a thin-layer plate (three spots for each standard and four for the test solution). After separation and color development, the somewhat light-sensitive spots were scanned as quickly as possible (filter 362 nm). The unknown amount of fructose was calculated from the average integrator responses by

Table I. Individual Monosaccharide Contents in Roasted and Instant Coffees. (All Data Expressed as % of Dry Extract)

Monose		Coffee			
		R & G		Instant	
Arabinose	min	n.d.[a]		0.40	
	avg	0.04%		0.97%	
	max	0.11		2.48	
Galactose	min	n.d.[a]		0.19	
	avg	<0.01		0.43	
	max	0.01		0.93	
Mannose	min			0.12	
	avg	n.d.[a]		0.35	
	max			1.05	
Ribose + xylose	min			0.04	
	avg	n.d.[a]		0.08	
	max			0.16	
		U. S.	German	U. S.	German
Glucose	min	n.d.[a]	n.d.[a]	0.02	n.d.[a]
	avg	0.26	0.03	0.16	0.03
	max	0.90	0.10	0.30	0.09
Fructose	min	n.d.[a]	n.d.[a]	0.18	n.d.[a]
	avg	0.16	0.05	0.29	0.04
	max	0.90	0.15	0.48	0.12
Number of samples analyzed		33	9	12	19

[a] Not detected (0.01–0.04% for mannose, depending on interference by fructose; <0.01% for all other sugars).

Table II. Monosaccharide Contents of R & G Coffee *vs.* Degree of Roast

Roasting degree	Rating		Monose contents, %[a]		
	U. S.	Germany	Glucose	Fructose	Arabinose
1	Too light	By far too light	1.07 1.39	1.61 1.90	0.10 0.13
2	Acceptable	Too light	0.24 0.08	0.48 0.11	0.11 0.07
3	Too dark	Acceptable	0.07 0.01	0.13 0.02	0.05 0.01
4	Much too dark	Too dark	n.d.[b] n.d.	n.d. n.d.	n.d. n.d.

[a] Based on soluble extract dry matter. [b] Not detected. For detection limits, see Table I.

linear extrapolation.

RESULTS AND DISCUSSION

42 roasted and 31 instant coffee samples of German and U. S. manufacturers were analyzed for their monose contents. The results are presented in Table I. The roasted coffee data include the results on five commercial liquid coffee concentrates. These were obtained by extraction at 100°. Their monose pattern and contents were not different from those of roasted coffee beans.

The occurrence of glucose and fructose in roasted coffees has been reported by Wolfrom *et al.* (1960) and Natarajan *et al.* (1955). According to Streuli (1970), Thaler claimed that arabinose is invariably present. Streuli himself could not confirm this result.

Our investigations have proven that small amounts of arabinose are present in most but not all roasted coffees. Occasionally a trace of galactose can be detected. Relatively high concentrations of glucose and fructose are found in most of the U. S. roasted coffee bean samples we examined, but only traces of these sugars were found in the German roasted coffee beans.

Table III. Estimated Extent of Hydrolysis of Coffee Polysaccharides to Monoses during Instant Coffee Manufacture

	Avg monosaccharide contents of instant coffee,[a] %	Avg monosaccharide contents of instant coffee based on R & G coffee[b] %	Avg polysaccharide contents of medium roasted coffee,[c] %	Avg percentage of polysaccharide hydrolysis[d]
Arabinose	0.97	0.485	0.8	60.6
Galactose	0.43	0.215	6.5	3.3
Mannose	0.35	0.175	14.2	1.2
Glucose	0.03[e]	0.015	5.5	0.3

[a] From Table I. [b] Assumed extraction yield of 50% from R & G coffee. [c] Thaler and Arneth (1969, Tables 1–3), based on green bean dry matter. [d] Ratio of monose contents (column 2) to polysaccharides (column 3). [e] The glucose value is based exclusively on German instant coffees (darker roast than U. S. coffees, see Table II) to exclude the influence of glucose originally present, or formed by hydrolysis of sucrose.

This is explained by the lighter roast usually given to American coffees as compared to German ones. Whereas in darker roasts the sucrose in the coffee bean is nearly completely destroyed, in lightly roasted coffee it is only partially hydrolyzed to its constituent sugars. In order to prove this assertion, two experimental blends of coffee beans were roasted to different degrees. In these coffees, glucose, fructose, and arabinose were detected and quantified (Table II).

The monosaccharide fraction of instant coffees consists of the sugars arabinose, fructose, galactose, glucose, mannose, ribose, and xylose. These findings confirm and extend the results of Thaler (1957), who reported, on paper chromatographic evidence, the presence of galactose, mannose and very small amounts of arabinose. Streuli (1970) detected arabinose (0.5–1%), smaller amounts of galactose, and occasionally traces of glucose and mannose.

We found that in many instant coffees arabinose is the dominating sugar. Galactose and mannose are further main constituents, whereas ribose and xylose are mere trace components. As for fructose and glucose, the results on instant coffee were analogous to the analytical results on the roasted coffee beans.

Streuli (1970) claimed that, on the basis of their monosaccharide contents, roasted and instant coffees can be clearly differentiated. Our investigations confirm Streuli's claim. However, it has to be mentioned that he did not support his claim by any experimental evidence. Galactose is the most characteristic indicator of difference since it is present in instant but virtually absent in roasted coffees. With the analytical separation technique used in the present investigation there is no interference by other sugars. Several earlier observations related to our present findings are summarized below.

The constituent monoses of coffee polysaccharides are: arabinose, galactose, glucose, mannose, rhamnose, and xylose (Thaler and Arneth, 1968). Fructose is present in coffee as part of the sucrose molecule. With the exception of rhamnose, which we could not detect, all these sugars occur as free monosaccharides in instant coffees. Ribose probably has been formed by epimerization of arabinose (Hough and Pridham, 1957). Likewise, fructose may in part stem from epimerization of mannose or glucose (Ohno and Ward, 1961).

Average contents of the main sugars occurring as poly-

Table IV. Monosaccharides Formed on Autoclave Extraction of R & G Coffees. (One-Step Extraction, Time Duration 3 hr)

Extraction temperature, °C	Ara	Gal	Man	Extract % (C)	Ara	Gal	Man
	% (C)[a]				% (E)[b]		
	C. arabica (Columbia)						
100	0.13	0	0	28.8	0.46	0	0
120	0.32	0.02	0	32.2	0.99	0.05	0
130	0.64	0.03	0.02	37.2	1.73	0.09	0.04
140	0.72	0.05	0.03	40.4	1.77	0.12	0.08
150	0.72	0.23	0.13	40.3	1.79	0.57	0.32
160	0.36	0.69	0.47	40.6	0.89	1.69	1.15
170	0.27	0.89	1.19	39.5	0.68	2.25	3.00
180	0.11	0.31	1.08	34.5	0.31	0.90	3.12
	C. robusta (Congo)						
100	0	0	0	32.6	0	0	0
120	0.14	0	0	35.1	0.40	0	0
130	0.19	0.02	0	39.1	0.49	0.06	0
140	0.77	0.12	0.04	47.9	1.60	0.24	0.08
150	0.88	0.32	0.08	45.7	1.93	0.71	0.19
160	0.59	0.53	0.33	46.1	1.28	1.16	0.71
170	0.20	1.30	1.45	43.2	0.46	3.00	3.36
180	0.04	0.23	1.00	36.3	0.11	0.64	2.76

[a] Based on R & G coffee dry matter. [b] Based on soluble extract dry matter.

saccharides in coffee beans are given by Thaler and Arneth (1969). Their separately presented data of water-soluble, digestible, and holocellulose polysaccharides in medium roasted coffee were summed up and the values of the different coffees investigated were averaged. From our own analytical data, average free monose contents of instant coffees were calculated. The ratio of monosaccharides content in instant coffee to polysaccharides content in roasted coffee, based on the assumption of a 50% extract yield, is an approximate measure of the extent of polysaccharide hydrolysis (Table III).

The different amounts, stabilities, and solubilities of coffee polysaccharides (Courtois *et al.*, 1963; Glomaud *et al.*, 1966; Thaler, 1957, 1959; Thaler *et al.*, 1968, 1969; Wolfrom and Anderson, 1967; Wolfrom and Patin, 1964, 1965; Wolfrom *et al.*, 1961) offer an explanation for the different monose contents of instant coffees. Glucan (cellulose) is the most stable toward hydrolysis, followed by (galacto-)mannan. The galactose chain of (arabino-)galactan is more accessible to hydrolysis while its arabino-furanoside side chains undergo cleavage quite readily.

A series of tests with a lab-scale autoclave served to provide the information about the influence of different extraction conditions on the composition of the monose fraction in particular. Coffees of different botanical origin and of different degrees of roast were exposed to different extraction temperatures for various lengths of time. Although, on a quantitative level, this is not comparable to the commercial coffee extraction process, nevertheless, qualitatively identical reactions could be expected to occur.

Two main differences exist between our procedure and the commercial process. First, the autoclave extracts have a much lower final concentration of soluble solids (≤3%, as compared to *ca.* 40% of the commercial extracts). Second, it is not possible to copy the negative temperature gradient of the commercial extraction. This latter obstacle was by-passed by splitting the temperature program. In one series the coffee was heated at a given temperature for the whole extraction time. In another one, the sample was exposed discontinuously to rising temperatures and, at each stage, fresh water replacing the coffee solution formed during the previous extraction step. In this series, the coffee samples were exhaustively extracted with hot water prior to the first autoclave treatment.

In low-temperature extracts, small amounts of glucose and fructose were detected. Again, glucose was found in extracts prepared at 180° or higher, most likely formed by hydrolysis of glucan (cellulose). Likewise, the amounts found remained low. Arabinose, galactose, and mannose, however, were liberated to considerable extents, depending on the extraction conditions. Their concentrations are given in the following tables (Tables IV and V), based both on coffee dry matter and dry extract.

Close examination of the data presented reveals that the liberation of arabinose starts below 120° and comes to an end at 140–160°. Formation of galactose and mannose is negligible below 150°. At temperatures of 170–180°, concentrations of galactose reach a maximum in extracts of the Arabica coffees. The Robusta coffee, however, yielded galactose at even higher temperatures. Likewise, mannose was liberated from all coffees still at 200° (Table V; the

Table V. Monosaccharides Formed on Autoclave Extraction of R & G Coffees. Stepwise Extraction at Rising Temperatures[a]

Total extraction time, hr	Extraction temperature, °C	Ara	Gal	Man	Extract % (C)	Ara	Gal	Man
		% (C)[b]				% (E)[c]		
		C. arabica (Columbia)						
1	120	0.08	0	0	30.3	0.26	0	0
2	140	0.50	0.02	0.02	40.8	1.22	0.05	0.05
3	160	0.77	0.13	0.13	52.0	1.48	0.25	0.25
4	180	0.79	0.22	1.18	63.5	1.24	0.35	1.86
5	200	0.79	0.22	1.59	68.0	1.16	0.32	2.34
		C. robusta (Congo)						
1	120	0.08	0	0	36.5	0.22	0	0
2	140	0.55	0.02	<0.01	48.4	1.14	0.04	0.01
3	160	0.90	0.15	0.10	60.1	1.50	0.25	0.17
4	180	0.92	0.30	1.25	70.8	1.30	0.42	1.77
5	200	0.92	0.48	1.52	74.4	1.24	0.65	2.04
		C. arabica (Salvador)						
1	120	0.04	<0.01	0	29.0	0.13	0.01	0
2	140	0.29	0.03	0.01	37.1	0.78	0.08	0.03
3	160	0.59	0.17	0.14	46.4	1.28	0.38	0.31
4	180	0.65	0.25	0.80	51.6	1.25	0.49	1.55
5	200	0.65	0.25	0.87	53.0	1.22	0.47	1.65

[a] The data shown for a given temperature represent the total amounts of sugars liberated up to that temperature. [b] Based on R & G coffee dry matter. [c] Based on soluble extract dry matter.

Table VI. Dependence of Monose Formation during Autoclave Extraction of R & G Coffee on the Degree of Roast

Degree of roast	Ara	Gal	Man	Extract	Ara	Gal	Man
	% (C)[a]			% (C)	% (E)[b]		
	C. arabica (Columbia)						
Light (2)[c]	1.08	0.25	0.18	40.9	2.64	0.62	0.43
Medium (3)	0.72	0.23	0.13	40.3	1.79	0.57	0.32
Dark (4)	0.46	0.19	0.09	41.2	1.15	0.45	0.22

[a] Based on R & G coffee dry matter. [b] Based on soluble extract dry matter. [c] For rating, see Table II.

respective data of Table IV suffer from rapid sugar decomposition; see below).

During one-step extraction (Table IV), considerable decomposition and formation of tar-like material occurs at temperatures above 140°. Thus, the amount of solubles yield extracted passes a maximum at this temperature. Decomposition of monosaccharides becomes obvious only at 180° when sugar concentrations decrease sharply, reaching zero values at 200°.

Discontinuous extraction at rising temperatures (Table V) largely prevents decomposition of solubilized coffee matter. (Industry has moved in this direction by flushing more water through the percolator extractors and removing lower solubles concentrations and higher solubles yields since 1965 (M. Sivetz).) High extract yields are obtained. Of the monoses formed, arabinose concentration reaches a plateau (based on coffee dry matter). As compared to one-step extraction, galactose concentrations remain low. Apparently, solubilized galactans are removed with the extracted material in this type of extraction, thus evading further hydrolysis.

Various coffees will yield widely different amounts of extract and monosaccharides under identical extraction conditions (Tables IV and V). However, these differences cannot be attributed to the botanical origin only. Arabicas and Robustas behave qualitatively in quite a similar way. The only exception found is the steeply rising galactose concentration of our high-temperature Robusta extracts. It appears as if this effect is due to hydrolysis of a cell wall galactan present in our Robusta coffee.

The different amounts of monosaccharides formed on extraction under hydrolytic conditions depend also on the degree of roast. Table VI shows the results of one-step extraction (3 hr, 150°) of coffee roasted to three different degrees. The light roast yielded roughly twice as much monoses as the dark roast.

It is interesting to note that in spite of the different absolute sugar amounts, the relative composition of the monose fraction changes very little with the degree of roast of the starting material. This ratio arabinose–galactose–mannose depends strongly on the extraction temperatures but is fairly independent of the coffee used (Tables IV and V).

Certainly, the extraction procedure plays an important part. In our model experiments, galactose values of high-temperature extracts are certainly too low in discontinuous extractions. In the commercial process, this discontinuity does not exist. On the other hand, in one-step extractions, arabinose concentrations of high-temperature extracts will be too low. Our extraction methods lack a negative temperature gradient, as was already mentioned above. Permanent high temperature thus leads to excessive decomposition. It should be a rational assumption that the actual fate of roasted coffee during the commercial extraction process is encircled by the results of our two temperature programs.

In practice, individual (Table I) and total monose contents of instant coffees may cover a wide range. Figure 3 shows, for a selected number of brands, the concentrations of the main and typical sugars arabinose, galactose, and mannose. The total contents of these three sugars were as low as 0.97 (no. 4) and as high as 4.15% (no. 6).

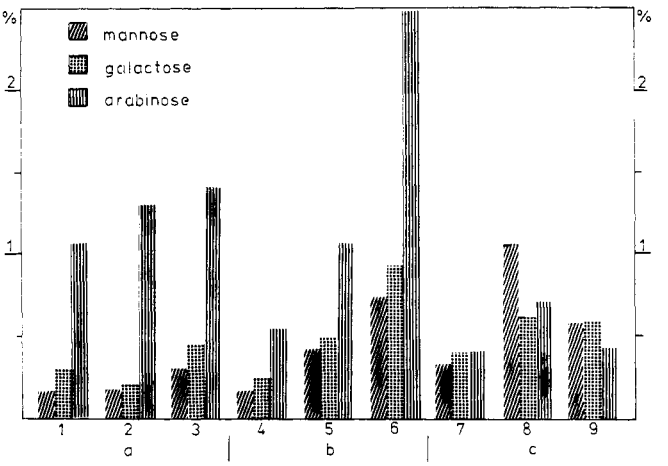

Figure 3. Arabinose, galactose, and mannose contents of a selected number of instant coffees. Classification into three groups: (a) arabinose ≫ galactose + mannose; (b) arabinose ≃ galactose + mannose; (c) arabinose ≃ galactose ≃ mannose. In the same order increasing hydrolysis of polysaccharides is likely to have taken place.

It can be seen from Figure 3 that the relative composition of the monosaccharide fraction, likewise, is subject to considerable variation. All instant coffees investigated can be arranged roughly into three groups: arabinose ≫ galactose + mannose (12 samples); arabinose ≃ galactose + mannose (12 samples); arabinose ≃ galactose ≃ mannose (7 samples). The stability differences of coffee polysaccharides suggest, in accordance with the above-mentioned experimental results, that this classification mainly reflects different coffee processing conditions. Maximum extraction temperatures are likely to increase in the order given above. At lower temperatures mainly arabinose is liberated. High processing temperatures will favor cleavage of galactans and mannans with simultaneous partial decomposition of previously formed arabinose.

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Influence of Storage on Composition, Amino Acid Content, and Solubility of Soybean Leaf Protein Concentrate

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Soybean Leaf Protein Concentrate (LPC) was prepared by either acid (LPC pI) or heat (LPC Δ) precipitation. Freshly prepared, freeze-dried LPC pI contained 1.55% moisture, 10.92% nitrogen, and 9.60% lipid, whereas LPC Δ contained 2.67% moisture, 11.80% nitrogen, and 8.19% lipid. With the exception of the limiting amino acid methionine, the amino acid profile compared favorably with the FAO reference protein. LPC Δ was virtually insoluble in water adjusted from pH 1.50 to 11. LPC pI was >60% soluble at pH 2.0 and 10.0 and above but <10% soluble from pH 3.5 to 6.8. Samples of both LPC preparations were stored for up to 24 weeks at 27° in the presence of oxygen. There was significantly more isoleucine, leucine, and lysine in LPC Δ, whereas LPC pI contained more glutamic acid, glycine, and histidine, irrespective of storage time. Methionine, glutamic acid, and tyrosine varied significantly during storage; only tyrosine, however, exhibited a linear trend. Solubility profiles were not influenced by storage.

Leaf Protein Concentrate (LPC) is one of several novel sources of protein which could be effectively used to combat protein malnutrition. Leaves are an especially appropriate source of protein in the tropics, where malnutrition is acute and vegetation flourishes.

The merits of LPC as a protein supplement for humans have been described previously (Kinsella, 1970; Pirie, 1970). Briefly, the favorable aspects of LPC include high yields, a wide variety of potential sources, simplicity of extraction and preparation, and good nutritive value (Buchanan, 1969b; Protein Advisory Group, 1970; Stahmann, 1968).

The yields of LPC have been investigated in Europe, India, and the United States (Akeson and Stahmann, 1966; Joshi, 1971; Lexander *et al.*, 1970). Yields of 1500 and 1670 kg of LPC/ha/yr have been reported for alfalfa (*Medicago sativa* L.) grown in India, and cocksfoot (*Dactylis glomerata* L.) grown in England, respectively (Arkcoll and Festenstein, 1971; Singh, 1969). More protein may be obtained from leaves per unit of land area than from any other agricultural commodity (Swaminathan, 1967).

The nutritive value of LPC has been evaluated by amino acid analyses, enzymatic and dietary studies. The amino acid profile of LPC indicates that it is nutritionally superior to most cereal and legume seed proteins including cottonseed and soybeans; it also compares favorably with most animal proteins except milk and eggs (Byers, 1971; Gerloff *et al.*, 1965; Hartman *et al.*, 1967). *In vitro* studies using pepsin-pancreatin showed that LPC was superior to beef and casein, equivalent to milk and lactalbumin, and inferior to egg protein (Akeson and Stahmann, 1965). *In vivo* studies with animals and humans were recently summarized (Singh, 1971; Woodham, 1971). LPC was shown to be an effective protein supplement for diets consisting mainly of either rice, raggi, wheat, or groundnuts (Doraiswamy *et al.*, 1969; Garcha *et al.*, 1971; Subba Rau and Singh, 1971; Sur, 1967). LPC has also been used as a milk extender for infants and young children (Waterlow, 1962).

As with most sources of novel protein, there are some problems relating to the acceptance of LPC. The major objections have been related to the green color of the concentrate and the development of undesirable odors and flavors during storage (Protein Advisory Group, 1970). The lipid fraction of LPC is held responsible for most of the deteriorative changes which occur when LPC is stored (Buchanan, 1969a). The lipid content of LPC ranges from 3.9 to 12.0% for the ether extractives and from 20 to 28% for the hot chloroform–methanol extracts (Byers, 1971; Oelschlegel *et al.*, 1969; Spencer *et al.*, 1971). It is noteworthy that from 53 to 79% of the fatty acids present are polyunsaturated and, thus, susceptible to oxidation under the appropriate conditions (Betschart, 1971; Lima *et al.*, 1965). The oxidation of the lipid fraction of LPC has, in fact, been described under a variety of conditions (Buchanan, 1969a,c; Lea and Parr, 1961; Shah *et al.*, 1967). Thus, the products and/or intermediates of lipid oxidation have been implicated in the formation of undesirable odors and flavors and the concomitant decrease in the nutritive value of stored LPC (Buchanan, 1969a; Kohler and Bickoff, 1971; Subba Rau and Singh, 1971).

Few studies have been conducted on the stability of stored LPC. Subba Rau *et al.* (1967) developed methods of increasing the shelf life of wet LPC. Buchanan (1969c) investigated the effects of storage upon LPC prepared according to the Pirie process (Morrison and Pirie, 1961).

The objective of the present study was to observe the

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