Table I. Mean[a] Panel Scores for Cantaloups Treated with Lindane Dust, Wettable Powder, or Emulsion Concentrate

Treatment	Flavor[b]		Odor[b]	
	Top half	Bottom half	Top half	Bottom half
Untreated, control	4.3	4.7	4.5	4.9
Lindane				
Dust	4.4	4.5	4.9	4.8
Wettable powder	3.8	3.6	4.5	4.5
Emulsion concentrate	2.8	3.0	3.5	3.7
Test difference[c]	0.8	0.6	0.4	0.5

[a] Mean for flavor of top halves of untreated cantaloups is based on 25 scores (5 judges, 5 scores per judge). All other means are based on 30 scores (5 judges, 6 scores per judge).
[b] 5 represents no off-flavor or off-odor; 1, very strong off-flavor or off-odor.
[c] Difference between two means is significant at 5% level when it equals or exceeds test difference [Duncan and Bonner test (*2*)].

Two days of preliminary training familiarized the judges with the natural flavor of the cantaloups and the form in which they were served.

The experimental design was a randomized block plan (*1*) which provided for two replications of both top halves and bottom halves of the four samples from each of the three field blocks. The design provided, therefore, six scores per judge for cantaloup top halves and six scores for cantaloup bottom halves for each treatment and for the untreated melons.

Taste-testing sessions were held in midmorning and mid-afternoon. At each session both top halves and bottom halves of the three treated samples and one untreated sample of melons representing a single field block, a total of eight samples, were rated. The samples were divided into two groups, top halves and bottom halves. The serving order was randomized, so that each group was served first four times. A 10-minute interval between the serving of the two groups of four samples allowed for recovery of the judges' sensory acuity.

Results and Discussion

Mean scores for flavor of untreated cantaloups and lindane-treated cantaloups show that development of off-flavors may be influenced by the form of lindane treatment, whether dust, wettable powder, or emulsion concentrate (Table I).

No significant difference was found between the flavor scores for the lindane dust-treated sample and the control sample. The mean flavor score for bottom halves of melons treated with lindane dust was significantly higher (flavor more natural) than the mean score for flavor of cantaloups treated with either emulsion concentrate or wettable powder. Also the mean score for top halves of melons treated with lindane dust was significantly higher than that for which emulsion concentrate was used. Both top and bottom halves of cantaloups grown with the use of lindane emulsion concentrate were scored significantly lower for flavor and odor than the untreated sample and melons receiving other forms of lindane treatment. Cantaloups treated with lindane emulsion concentrate were said by panel members to have a "musty" flavor more frequently than other treated samples as shown at right above:

Frequency of Occurrence of "Musty" Flavor

Control, %	Dust, %	Wettable Powder, %	Emulsion Concentrate, %
14	10	44	58

The effect of lindane wettable powder on the flavor of melons was less definite. Bottom halves of samples treated with lindane-wettable powder were significantly poorer in flavor than bottom halves of untreated samples. The top halves of lindane-wettable powder-treated melons also received a mean flavor score which was lower, although not significantly so, than the mean flavor score received by the top halves of untreated cantaloups.

Mean scores for top and bottom halves of the three treated samples were in agreement, indicating that the two portions of the melons were similarly affected by the lindane treatment.

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COFFEE CONSTITUENTS

Carbohydrates of the Coffee Bean

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THE chemistry of the coffee bean roasting process is not well understood, but is undoubtedly concerned in part with its carbohydrate content, because this amounts to 50 to 60% of the green bean. The literature on the carbohydrates of the coffee bean is incomplete and is often conflicting.

In the present work the authors (*14, 17–19*) have determined the free sugars present in green and roasted coffee. The mixed polysaccharide fractions of green and roasted coffee have been isolated. Derivatives of the sugars present in the polysaccharide fractions of green coffee have been isolated and a quantitative analysis of the sugars present is reported.

Green Coffee Bean

Coffee beans used in this investigation were grown on Fazenda Limeira (210, 000 trees), Morais Sales, Brazil, at an altitude of 631 meters, in Massapé soil (clayey earth), and were classified as Santos 4's, the mildest or least harsh to taste of the Brazilian coffees.

Chromatographic Methods

Flowing-type chromatography on clay columns was employed for the crude fractionation of the ethanol–water (80 to 20) extracts of green and roasted coffee.

The carbohydrates of green and roasted Santos coffee were identified and measured. The soluble sugars of green coffee were sucrose, 6 to 7%, and a trace of glucose; those of roasted coffeee were sucrose, glucose, and fructose. The holocellulose fractions of green and roasted coffee were isolated. The 10% potassium hydroxide–insoluble holocellulose of green coffee was hydrolyzed by a new method employing anhydrous sulfuric acid. It contained the following ratio of sugars: L-arabinose, 1; D-galactose, 2; D-glucose, 2; D-mannose, 6. Little, if any, lignin, cellulose, or pectin was found. The holocellulose was partially solubilized on roasting. The arabinose was easily acid-hydrolyzable. Other coffee constituents identified and estimated were caffeine, trigonelline, caffeic acid, chlorogenic acid, "isochlorogenic" acid, and 10 amino acids. The free amino acids disappeared on roasting. A method was developed for locating caffeine on chromatograms.

For paper chromatography of simple sugars, the descending method was used, according to Jermyn and Isherwood (*7*) or Quick (*15*). The spray reagent was either *p*-anisidine hydrochloride in water-saturated 1-butanol (2%, w./v.) or aniline (0.93 gram), and phthalic acid (1.66 grams) in water-saturated 1-butanol (100 ml.).

Electrochromatography was accomplished in borate buffer (ca. pH 10) at 800 volts and 10 to 15 ma. The same spray reagents were used as in paper chromatography.

Paper chromatography of the amino acids was effected by the two-dimensional, ascending method (*5*) using 0.2% of ninhydrin in water-saturated 1-butanol as a spray reagent.

Techniques were developed for the identification of caffeine on paper chromatograms and clay columns. The paper or electrochromatogram was dried and dipped in an acidified ether solution (5 drops of concentrated hydrochloric acid per 100 ml.) of pyrene (0.01%). Under ultraviolet light, the caffeine appeared as a spot of quenched fluorescence on a yellow-white background. The limit of sensitivity was about 20 γ of caffeine in a 15-mm.-diameter spot. It has been found (*25*) that a large number of purines quench the fluorescence of acidified aqueous solutions of various polynuclear aromatic substances. To detect caffeine on clay, the column was streaked with slightly acidified bromothymol blue indicator. The caffeine zone appeared as a blue section on the yellow streak.

Extraction of Soluble Sugars of Green Coffee Bean. Ground, green coffee bean (500 grams, 10% moisture, 80-mesh) was placed in quart Mason jars (50.0 grams per jar) and covered with 100 ml. of absolute ethanol. The contents of the jars were boiled for 2 to 3 minutes and the following additions were made to each jar: 150 ml. of ethanol-water (40 to 20 weight ratio), 20 ml. of water, 29 ml. of ethanol, and 0.5 gram of calcium carbonate. This made a total of 300 ml. of ethanol-water (80 to 20) per 50-gram sample. The contents of each jar were boiled 1 to 2 minutes and the jars were sealed and stored for 70 hours at 20° to 30° C. to effect extraction.

The mixture was filtered and the residue washed with 1 liter of 80% ethanol. The filtrate was concentrated to 100 ml. under reduced pressure at 40° to 45° C. The viscous amber sirup was dissolved in 800 ml. of methanol-ethanol (3 to 1) and adjusted to 2000 ml. with absolute methanol; only a trace of material did not dissolve.

ANALYSIS. Nonvolatile solids, made up 90.08 grams or 20% of dry weight of the original bean.

Fractionation of Ethanol-Water (80 to 20) Extract of Green Coffee Bean. An amount of 1900 ml. of the adjusted extract was added to the top of an 11 to 12 × 7 to 9 cm. diameter bed of 250 grams of a mixture of Florex XXX (Floridin Co., Warren, Pa.) and Celite (No. 535, Johns-Manville Co., New York, N. Y.) contained in a 2-liter pharmaceutical percolator. The adsorbent bed was prewashed with 1 liter of 95% ethanol and the column was developed by successive percolations of the developers shown in Table I.

The ethanol-water (95 to 5) fraction was concentrated slightly and then diluted to 2000 ml. Crystallization of crude sucrose was induced in four 200-ml. aliquots of this fraction. The product was recrystallized from ethanol; melting point 189–90° C., $[\alpha]_D^{27}$ + 67° [*c* (concentration, grams per 100 ml. of solution), 6.468, water] accepted values: 188° C. and +66.5°, respectively.

The supernatant liquid from the direct crystallization of sucrose was concentrated to a thick sirup and transferred with water to a continuous liquid-liquid extractor. The aqueous solution was extracted for 20 hours with ethyl ether. The aqueous solution which remained was freed of ether by heating at 45° to 50° C. under reduced pressure and was then lyophilized. The residue was a nearly white, crystalline material; yield 13.7 grams. Five grams of this substance were dissolved in 100 ml. of water and extracted for 20 hours with benzene in a continuous liquid-liquid extractor. The extract was freed of benzene by heating at 60° to 70° C. under reduced pressure and lyophilized. The white, crystalline residue was dissolved in 10 ml. of 95% ethanol by heating. On cooling to 30° C., white needles of caffeine formed; yield 0.124 gram, melting point 234–6° C., accepted value: 235° C. On further cooling to −25° C. a second crop of crystals was recovered; yield 0.305 gram, melting point 231–5° C.

The ether extract was concentrated to a brown fatty residue; yield 4.89 grams.

A 1000-ml. aliquot of the ethanol-water (95 to 5) fraction was concentrated to 150 ml. and extracted for 8 hours with benzene in a liquid-liquid extractor. The aqueous solution remaining after extraction was heated at 45° to 48° C. under reduced pressure to remove dissolved benzene and was then adjusted to 250 ml. with distilled water. Two 70-ml. aliquots of this solution were lyophilized. The resulting solids were dissolved in 15 ml. of absolute methanol to effect crystallization of sucrose; yield 4.2 grams for each 70-ml. aliquot; melting point 174° to 195° C., $[\alpha]_D^{32}$ +45.16° (*c* 4.53, water). The physical constants indicated that the crystals were impure. Electrochromatograms showed the presence of sucrose, chlorogenic acid, and a trace of caffeic acid. Calculations based on the assumption that chlorogenic acid, $[\alpha]_D$ −38°, was the only impurity and that the rotations were additive indicated that the crystals were 79% sucrose and 21% chlorogenic acid.

The mother liquors from the crystallization of sucrose were diluted to 200 ml. with 95% ethanol. A 10-ml. aliquot of this solution, diluted 1000 times, showed an absorbance at 324 mμ of 0.818 on a Beckman Model DU spectrophotometer. The chlorogenic acid content was calculated by the method of Moores, McDermott, and Wood (*13*).

Additional substances present in the ethanol-water (95 to 5) fraction were

Table I. Fractionation of Material from an Ethanol-Water (80:20) Extract of Green and Roasted Coffee Beans on Florex XXX[a] with Successive Ethanol-Water Developers

Substances Identified	Green Coffee			Roasted Coffee	
	Developer 1[b]	Developer 2[c]	Developer 3[d,e]	Developer 1[b]	Developer 2[c,f]
Sucrose	5.5[g]	+		+	
Chlorogenic acid	5.1	1.0		+	
Caffeine	0.7			+	+
Fats	2.9				
Amino acids	Trace[h]				
Glucose	Trace			+	
"Isochlorogenic" acid	+[i]	+		+[i]	+[i]
Caffeic acid	+	+		+[i]	+[i]
Trigonelline		+	+		+
Glycine		+			
α-Alanine		+	Trace		
γ-Aminobutyric acid		+	Trace		
Proline		+			
Valine		+			
Leucine		+			
Tyrosine		+			
Aspartic acid			+		
Glutamic acid			+		
Serine			+		
Asparagine			+		
Fructose				+	

[a] Fuller's earth clay, 5 parts Florex XXX to 1 part Celite No. 535 (weight ratio).
[b] Developer 1. 5 liters of ethanol-water (95 to 5); eluent contained 17.8% of the dry, green coffee and 12.6% of the dry, roasted coffee.
[c] Developer 2. 6 liters of ethanol-water (80 to 20); eluent contained 2.3% of the dry, green coffee and 2.4% of the dry, roasted coffee.
[d] Developer 3. 5 liters of ethanol-water (50 to 50); eluent contained 0.6% of the dry, green coffee and 1.3% of polymeric material from the dry, roasted coffee.
[e] Developing the column of green coffee finally with 4 liters of water (eluted 0.3%) followed by 3 liters of 0.1*N* hydrochloric acid (eluted 0.1%), and 4 liters of 0.5*N* hydrochloric acid, resulted in material which could not be separated by chromatography.
[f] Developing the column of roasted coffee finally with 4 liters of water (eluted 0.4%) followed by 6 liters of 0.1*N* hydrochloric acid resulted in material which could not be separated chromatographically.
[g] Percentage basis dry bean.
[h] Faint spot on a paper chromatogram.
[i] Definite spot on a paper chromatogram.
[j] Either one or both were present.

dentified by paper chromatography (Table I). Attempts to prepare a concentrate of glucose by precipitation of the chlorogenic acid with lead acetate resulted in the complete loss of glucose.

The 6 liters of the ethanol-water (80 to 20) fraction of the green coffee bean were concentrated and then diluted to 100 ml. A 60-ml. aliquot of this solution was lyophilized. An amount of 200 mg. of this lyophilized material was analyzed for chlorogenic acid by the ultraviolet absorption method (*13*). The measured absorbance was 0.458 at 324 mμ.

The material of the ethanol-water (80 to 20) fraction was chromatographed on paper simultaneously with known substances (Table I). Employing the two-dimensional, ascending method of Consden, Gordon, and Martin (*5*), the amino acids listed in Table I were identified—comparisons were made with simultaneously run known substances.

Chlorogenic acid was crystallized from 4.6 grams of solids from the ethanol-water (80 to 20) fraction by the method of Moores, McDermott, and Wood (*13*); yield 48 mg.; melting point 203° C., $[\alpha]_D^{26}$ −47.7° (*c* 0.2, water), accepted values: 206–8° C., and −38°, respectively.

Paper chromatography of the ethanol-water (50 to 50) fraction indicated the presence of trigonelline and several amino acids which were rechromatographed and identified as described above (Table I). Paper chromatograms of the water and the acid fractions gave poor resolution and no compounds could be identified. "Isochlorogenic" acid was isolated as a sirup from the green coffee bean by the method of Barnes, Feldman, and White (*1*) and was characterized chromatographically.

Roasted Coffee Bean

Green coffee beans of the type described above and which had been given a light roast were used in this part of the work.

Extraction of Soluble Sugars of Roasted Bean. Ground, roasted coffee beans (500 grams, 5.4% moisture, 80-mesh) were shaken with 1 liter of petroleum ether (boiling point 30° to 60° C.) and filtered. The residue was extracted with ethanol-water (80 to 20) at 25° to 35° C. for 6 days in the dark. The extract was filtered, concentrated, and diluted to 2000 ml. with methanol and ethanol as with the green coffee extract.

Analysis. Nonvolatile solids made up

Table II. Compilation of Data on Fractionation of Green and Roasted Coffee, Dry Weight Basis

Fraction Extracted[a]	Green Coffee, %	Roasted Coffee, %
Ethanol-water (80:20)	15.9	16.2
Benzene-ethanol (2:1)	17.4	16.0
Water	15.7	12.8
Ammonium oxalate	3.0	3.7
Acidified sodium chlorite	6.3	8.2
KOH, 10%	4.9	8.6
Alkali-insoluble holocellulose, final residue	36.8	34.5
Total	100.0[b]	100.0

[a] Extractions were made successively from top to bottom.
[b] Adjusted from a 97.6% actual summation for comparative purposes.

80.0 grams or 16.9% of the dry weight of the roasted bean.

Fractionation of Ethanol-Water (80 to 20) Extract of Roasted Coffee Bean. An amount of 1900 ml. of the 2000-ml. roasted coffee extract was fractionated on a clay column, analogous to that used for the green coffee extract with the developers shown in Table I. The hydrochloric acid fraction was light yellow-brown; all the other fractions were dark brown.

The ethanol-water (95 to 5) fraction was adjusted to 2000 ml. with absolute ethanol. Four 200-ml. aliquots were used in an unsuccessful attempt to crystallize sucrose.

A paper chromatogram of the ethanol-water (95 to 5) fraction was developed and observed under ultraviolet light. The presence of caffeine was shown by developing a chromatogram for 11 hours and was detected as described above.

The ethanol-water (80 to 20) fraction was concentrated to 200 ml. A chromatogram of this solution was developed and viewed under ultraviolet light to indicate the presence of trigonelline, chlorogenic and "isochlorogenic" acids (or caffeic acid or both).

No amino acids were found in the ethanol-water (80 to 20) fraction of roasted coffee bean by the method (*5*) used to identify the amino acids in the green coffee bean.

Paper chromatograms of the ethanol-water (50 to 50), water, and acid fractions showed no resolution. These fractions were not further investigated.

Polysaccharide Fractions of Green Coffee

Ground, green coffee beans (2400 grams, 5.6% moisture) were extracted with ethanol-water (80 to 20), as described above. The insoluble residue

was then extracted successively with benzene-ethanol, water, three times at 20° to 25° C., and twice with 12 liters of 0.5% ammonium oxalate at 90° C. (Table II).

The investigation of the ethanol-water (80 to 20) extract has been described above. The benzene-ethanol extract contained mostly lipides and was not examined further.

An amount of 9.5 liters of the first water extract (neutral to litmus) was poured with stirring into 22 liters of absolute ethanol (final concentration 70% ethanol). The flocculent cream-colored precipitate formed was recovered by filtration and lyophilized; yield 72.7 grams of a gray powder (3.8% ash as sulfate) or 3.5% of the original bean on a dry weight basis. The 70% alcohol-insoluble powder gave a negative ferric chloride test (for phenols), a positive ninhydrin test, and a positive Molisch test. Hydrolysis with 1*N* sulfuric acid for 8 hours (homogeneous solution) yielded arabinose and galactose by paper chromatography.

The filtrate from the 70% ethanol precipitation was concentrated and lyophilized; yield 213.0 grams of dense yellow solids which were not further examined. This amounted to 10.2% of the original bean on a dry weight basis. The work of Underwood and Deatherage (*23*) would indicate that this fraction was largely protein in nature.

This water extraction was repeated twice in the same manner; yield 21.8 grams (1.1% of tan colored) and 8.7 grams (0.4% of light green) solids, respectively. The products gave positive ferric chloride, ninhydrin, and Molisch tests.

The material from the first 0.5% ammonium oxalate extract gave a negative ferric chloride test, a strongly positive ninhydrin test, and a positive Molisch test. The residue from the first ammonium oxalate extraction was extracted once more with 0.5% of ammonium oxalate at 90° ± 3° C. The combined ammonium oxalate extracts were concentrated under reduced pressure and lyophilized; yield 21.4 grams after subtraction of its ammonium oxalate content. This light fluffy solid gave a negative ferric chloride test, a strongly positive ninhydrin test, and a positive Molisch test.

An amount of 150 mg. of material from the first ammonium oxalate extract was hydrolyzed for 2 hours with 1% sulfuric acid. A paper chromatogram of the hydrolyzate indicated galactose, mannose, arabinose, rhamnose or apiose [apiose is almost identical to rhamnose in chromatographic behavior in ordinary solvents (*2*)], and two, slow-moving spots, one of which was probably galacturonic acid. Comparisons were made with simultaneously run known substances. Mannose and arabinose have nearly identical R_f values in this developer and further confirmation of these two sugars was obtained by electrochromatography, by which method mannose and arabinose are readily separated.

Coffee Holocellulose

The holocellulose was prepared essentially according to the modification by Whistler and associates (*26*) of the procedure of Wise and coworkers (*27*). The holocellulose, a nearly white powder, gave a negative ferric chloride test for tannin, a negative ninhydrin test, and a strong positive Molisch test.

Extraction of Holocellulose with Alkali. Coffee holocellulose (25.0 grams) was stirred for 24 hours in 250 grams of 10% potassium hydroxide at 25° ± 1° C. under nitrogen. The mixture was filtered through a glass filter and the residue was washed with 250 grams of 10% potassium hydroxide and 300 ml. of water. The combined filtrate and wash liquors were adjusted to pH 5 with 50% acetic acid and centrifuged; yield 0.15 grams of gray solids, hemicellulose-A fraction. The centrifugate was diluted with three volumes of absolute ethanol and centrifuged; yield 1.77 grams of a cream-white powder, hemicellulose-B fraction. The hemicellulose-A and -B fractions were each hydrolyzed with 1*N* sulfuric acid and the neutralized hydrolyzates were paper-chromatogrammed. Rhamnose (or apiose) (*2*), xylose, arabinose, galactose, a trace of mannose, and several slow-moving, inconclusively identified, spots were detected in the hydrolyzate of hemicellulose-B, whereas no sugar spots could be detected in the hydrolyzate of hemicellulose-A.

The 10% potassium hydroxide–insoluble residue was an almost pure white, granular powder; yield 20.3 grams.

The amounts of apparent lignin in several of the coffee fractions were determined by the method of Ritter and Barbour (*16*). The results were: original bean, 2.40%; holocellulose, 1.80%; 10% potassium hydroxide–insoluble holocellulose, 0.35%. It is doubtful whether these values represent true lignin

Polysaccharide Fractions of Roasted Coffee

For comparison purposes a quantity of roasted coffee was carried through the same fractionation procedure as that described above for the isolation of the polysaccharide fractions of green coffee (Table II).

Characterization of 10% Potassium Hydroxide–Insoluble Holocellulose of Green Coffee Bean

One gram of 10% potassium hydroxide–insoluble holocellulose was hydrolyzed with 25 ml. of 72% sulfuric acid, according to the procedure of Monier-Williams (*12*). The sirup resulting from the hydrolysis was chromatographed on paper using the method of Quick (*15*). The paper was sprayed with aniline phthalate reagent and four well-resolved spots corresponding to glucose, galactose, mannose, and arabinose were detected.

Extraction with Cuprammonium Solution. Ten grams of 10% potassium hydroxide–insoluble holocellulose were stirred into 250 ml. of cuprammonium solution, prepared by the method of Launer and Wilson (*9*). The flask was tightly stoppered and kept for 8 hours at 10° to 20° C. and for 20 hours at 25° to 30° C. The mixture was filtered and the residue was neutralized with 18*N* sulfuric acid, washed with water, and dried at room temperature; yield 7.0 grams. The filtrate was neutralized with 18*N* sulfuric acid in an ice bath and yielded a white, flocculent precipitate which was collected by filtration and dried in air at room temperature; yield 1.1 grams.

An amount of 15 mg. of cuprammonium-soluble material was refluxed as a homogeneous solution for 24 hours in 1*N* sulfuric acid. Electrochromatography of the resulting hydrolyzate showed a strong mannose spot, a glucose spot, and a weak galactose spot.

Partial Hydrolysis with 1.5% Sulfuric Acid. One gram of 10% potassium hydroxide–insoluble holocellulose was mixed with 50 ml. of boiling 1.5% sulfuric acid, refluxed for 5 minutes, and rapidly filtered through a glass filter.

The unhydrolyzed residue was returned to the reflux flask with a fresh portion of boiling 1.5% sulfuric acid. The hydrolysis procedure was repeated several times, gradually increasing the contact time.

Paper chromatograms of the resulting hydrolysis sirups showed that only arabinose was removed upon refluxing for 5 minutes. All of the arabinose was removed after 15 minutes of reflux, but in this aliquot some galactose was also present. No mannose or glucose was removed even after a cumulative refluxing time of 6 hours.

The 10% potassium hydroxide–insoluble holocellulose of roasted coffee was also selectively hydrolyzed with 1.5% sulfuric acid, as described above. The arabinose was again readily removed from this material along with a trace of mannose and a small amount of galactose.

Derivatives of Constituent Sugars

A 1.0200-gram sample of 10% potassium hydroxide–insoluble holocellulose of green coffee was refluxed for 5 minutes

in 1.5% sulfuric acid and filtered quickly. The filtrate was neutralized with Duolite A-4 ion exchange resin (Chemical Process Co., Redwood City, Calif.). An electrochromatogram of the resulting sirup showed only the presence of arabinose.

The tetra-*O*-acetyl-L-arabinose diethyl dithioacetal derivative was then synthesized from the sirup by the procedure of Wolfrom and Karabinos (*28*); melting point 76.0–6.2° C. corrected, unchanged on admixture with authentic material, $[\alpha]_D^{22}$ −26.4° (*c* 1.54, chloroform); accepted values: 79–80° C. and −29.9°, x-ray powder diffraction data (interplanar spacing, CuK_α radiation; relative intensity, estimated visually; vs, very strong; m, medium; w, weak; vw, very weak) (identical with that of authentic material): 13.59 vw, 9.61w, 7.13vs, 6.19vw, 5.54vw, 4.82m, 4.51vw, 4.09s, 3.88m, 3.72vw, 3.59w, 3.40vw, 3.22vw, 2.96vw, 2.83vw, 2.67vw, 2.51vw, 2.42vw.

Ten grams of 10% potassium hydroxide–insoluble holocellulose of green coffee were refluxed for 15 minutes in 250 ml. of boiling 1.5% sulfuric acid and filtered. The undissolved residue was hydrolyzed with 72% sulfuric acid according to the procedure of Monier-Williams (*12*).

A 5.2-gram sample of the brown hydrolysis sirup was acetylated with anhydrous sodium acetate and hot acetic anhydride and poured into water. A chloroform extract of the water was evaporated to a sirup. A 1.0-gram sample of this acetate sirup in 100 ml. of benzene was added at the top of a column (250 × 75 mm. in diameter) of 5 parts of Magnesol (Westvaco Chemical Division, Food Machinery and Chemical Corp., South Charleston, W. Va.) and 1 part of Celite No. 535 (weight ratio), prewet with 150 ml. of benzene. The column was developed with 2500 ml. of benzene–*tert*-butyl alcohol (200 to 1 volume ratio). Two zones were located by streaking the extruded column with permanganate indicator (1% potassium permanganate in 2.5*N* sodium hydroxide). The two zones were numbered from top to bottom and sectioned. The sugar acetate in each was removed by extraction with acetone and evaporated to sirups.

The material from zone 2 crystallized from ethanol as penta-*O*-acetyl-β-D-glucopyranose; melting point 129–30° C. unchanged on admixture with authentic material $[\alpha]_D^{30}$ +6.6° (*c* 3.4, chloroform); accepted values: 135° C. and +3.8°, respectively.

The material from zone 1 crystallized from ethanol as penta-*O*-acetyl-β-D-mannopyranose; melting point 112–13° C. unchanged on admixture with authentic material, $[\alpha]_D^{31}$ −21° (*c* 3.3, chloroform); accepted values: 117–18° C. and −25.2°. D-Mannose was also isolated from the hydrolyzate sirup of 10% potassium hydroxide–insoluble holocellulose of green coffee as D-mannose phenylhydrazone; melting point 186–8° C. unchanged on admixture with authentic material, $[\alpha]_D^{27.5}$+23° ± 4° C. (*c* 0.1, pyridine); accepted values: 199–200° C. and +26.3°, respectively.

A major portion of the sirup from the hydrolysis of 10% potassium hydroxide–insoluble holocellulose of green coffee was added in a streak to an 18.25 × 22.5 inch Whatman No. 3 filter paper. Nine such papers were prepared and developed with 1-butanol–pyridine–water (10:3:3) for 98 hours. Indicator strips were cut from each side and from the center, and sprayed with aniline phthalate. The paper chromatograms were pieced together and the zones of the unsprayed portions corresponding to the movement of galactose were cut out and pulverized in 1 liter of distilled water. The mixture was filtered through Whatman No. 1 filter paper and the filtrate was evaporated to a sirup. A very small portion of the sirup was electrochromatographed and showed only the presence of galactose. The remainder of the sirup was acetylated with anhydrous sodium acetate and hot acetic anhydride and poured into 100 ml. of ice and water. A chloroform extract was concentrated to a sirup. The sirup was dissolved in hot, absolute ethanol and upon cooling and seeding with penta-*O*-acetyl-β-D-galactopyranose, crystallization occurred; melting point 140.9–1.3° C. unchanged on admixture with authentic material, $[\alpha]_D^{24}$+26° ± 4° C. (*c* 0.97, chloroform); accepted values: 142° C. and +25°, respectively. X-ray powder diffraction data, identical with that of authentic material, were as reported by Wolfrom and associates (*30*).

Quantitative Determination of Constituent Sugars in 10% Potassium Hydroxide–Insoluble Holocellulose

The amount of pentose (6.7%) was first determined by the 2-furaldehyde method.

A direct quantitative determination of mannose was obtained by hydrolyzing with 72% sulfuric acid, according to the procedure of Monier-Williams (*12*) and precipitating the mannose as phenylhydrazone. A value of 46.3% mannose was so obtained.

Considerable difficulty was encountered in attempting to determine the amounts of galactose and glucose in this fraction of the green coffee bean by direct chemical procedures. It was also evident that the 72% sulfuric acid method of Monier-Williams (*12*) did not result in complete hydrolysis, because the material did not dissolve completely without considerable degradation. After neutralization and concentration of the hydrolyzate, polysaccharide material was precipitated from the sirup by the addition of absolute methanol. A quantitative determination of the constituent sugars was finally obtained as described below.

A 3.0-gram sample of holocellulose of the green coffee bean was extracted with 10% potassium hydroxide for 24 hours at 25° ± 2° C. The mixture was filtered and the residue washed free of base with water. The 10% potassium hydroxide–insoluble holocellulose was lyophilized.

A 2.0295-gram sample of this freshly lyophilized material was added to 45.0 grams (24.5 ml.) of anhydrous sulfuric acid (melting point 7° to 9°) in a round-bottomed flask submerged in a sodium chloride–ice-water bath at −2° C. After 54 hours the 10% potassium hydroxide–insoluble holocellulose had dissolved with the exception of a few, very small particles. The solution was dark in color. Water was then added dropwise from a capillary tube at such a rate that the temperature was kept below 10° C. The solution was strongly stirred during the addition of water. The hydrolysis solution was diluted to 1500 ml. to give a 3% concentration of sulfuric acid. The 1500-ml. solution was light amber in color and no insoluble material could be detected.

The hydrolysis solution was refluxed and 2-ml. aliquots were removed after 6, 12, 15, and 18 hours. Because the Somogyi (*21*) reducing values of the 15- and 18-hour aliquots were constant, it was considered that hydrolysis was complete. The solution was neutralized with a saturated aqueous solution of barium hydroxide and the resulting precipitate of barium sulfate was removed by centrifugation. The supernatant liquid was made slightly acid to methyl red with 0.1*N* sulfuric acid and concentrated to 600 ml. under reduced pressure at 40° to 50° C. This 600-ml. portion was carefully neutralized to litmus paper with barium hydroxide and filtered under gravity. The filtrate was decolorized with a very small amount of carbon and after filtration to remove the carbon, was concentrated somewhat and then diluted to 250 ml. A 200-ml. aliquot was concentrated as above to a sirup which was refluxed with 200 ml. of absolute methanol. This resulted in a white precipitate of inorganic salts that was removed by filtration. The methanol filtrate was concentrated to a sirup which dissolved in cold, absolute methanol with no resulting precipitate. The solution was again concentrated to a sirup which was dissolved in water and diluted to 200 ml.

Quantities of this solution ranging from 0.015 to 0.030 ml. were applied from a microburet (Aloe Scientific Division, A. S. Aloe Co., St. Louis, Mo.) to paper chromatograms which were

developed according to the procedure of Quick (*15*). The chromatograms were sprayed with aniline phthalate reagent, air-dried for 15 minutes, and then heated in an oven at 110° to 115° C. for 15 minutes. The densities of the resulting spots were read as quickly as possible with a Photovolt Electronic Densitometer, Model 525 (Photovolt Corp., 95 Madison Ave., New York 16, N. Y.) using ordinary white light and a slit width of 6 mm. The amounts of sugars corresponding to the densities of the spots were then determined from standard curves prepared from known sugar solutions in which logarithms of the concentrations were plotted *vs.* densities. This technique closely followed that of McFarren, Brand, and Rutkowski (*11*) for the quantitative determination of sugars on filter paper chromatograms by direct photometry.

The results of the quantitative determinations in percentage of the theoretical hydrolysis yield were as follows: D-glucose, 17.8%; D-mannose, 48.5%; D-galactose, 14.8%; L-arabinose, 2.8%; total yield 83.9%. This sum of the separate sugar percentages compares favorably with the 85.4% yield of a total solids analysis of the same solution.

Because the strongly acidic conditions used for hydrolysis degraded arabinose, this sugar was quantitatively determined in a separate sample by refluxing 1.0297 grams of 10% potassium hydroxide–insoluble holocellulose with 50 ml. of 1.5% sulfuric acid for 20 minutes. The mixture was filtered and the filtrate was neutralized and concentrated to a sirup, as described above. The amount of arabinose in the sirup was quantitatively determined by paper chromatography and direct photometry as described above; yield 6.0%.

The results of the quantitative determinations in percentage of the theoretical hydrolysis yield, using 6.0% for arabinose, were as follows: D-glucose, 17.8%; D-galactose, 14.8%; D-mannose, 48.5%; L-arabinose, 6.0%. Total recovery, 87.1% of the theoretical. Correcting these values to 100.0% resulted in: D-glucose, 20.4%; D-galactose, 17.0%; D-mannose, 55.7%; L-arabinose, 6.9%. Considering arabinose as 1 this resulted in a molar ratio of: L-arabinose, 1.0; D-glucose, 2.2; D-galactose, 1.8; and D-mannose 6.2.

The 10% potassium hydroxide–insoluble holocellulose of roasted coffee was hydrolyzed with anhydrous sulfuric acid, as described above for the hydrolysis of green coffee. Paper chromatograms developed simultaneously with known compounds showed the presence of arabinose, glucose, mannose, and galactose.

Results and Discussion

The procedure used in the fractionation of the ethanol-water (80 to 20) extract of green coffee was similar to the clay column techniques developed by Wolfrom and coworkers (*3, 10*).

Table I includes the data obtained on the fractionation of the ethanol-water (80 to 20) extract of the green coffee bean on a clay column.

Sucrose was identified by crystallization from the ethanol-water (95 to 5) fraction. A small amount of sucrose was also identified in the ethanol-water (80 to 20) eluent from the clay column by paper chromatography, but it resisted crystallization. It may be estimated that the green coffee bean contains 6 to 7% sucrose. This agrees with the work of Ewell (*6*) and of Slotta and Neisser (*20*).

Chlorogenic acid was identified by crystallization from the ethanol-water (80 to 20) eluent from the clay column. The green coffee bean contains a total of 6.1% of chlorogenic acid.

The presence of a trace of glucose in the ethanol-water (95 to 5) fraction of the ethanol-water (80 to 20) extract of green coffee was indicated by paper chromatography. The presence of caffeic acid and "isochlorogenic" acid was indicated by paper chromatography. "Isochlorogenic" acid was identified by comparison with a known preparation isolated by the procedure of Barnes, Feldman, and White (*1*).

The ethanol-water (80 to 20) fraction (green coffee) from the clay column contained the substances indicated in Table I. The amino acids which were found were not in complete agreement with the report of Underwood and Deatherage (*23*). However, the authors extracted the free amino acids with ethanol-water (80 to 20) whereas Underwood and Deatherage undoubtedly hydrolyzed some proteins with their 6*N* hydrochloric acid extraction. The ethanol-water (50 to 50) fraction (green coffee) from the clay column contained the substances shown in Table I.

Table I includes also data obtained on the fractionation of the ethanol-water (80 to 20) extract of the roasted coffee bean on a clay column. The investigation of the roasted coffee extract was complicated by dark brown decomposition products. There was no evident fractionation of the brown-colored substances by the clay column, and on paper chromatograms yellow-brown streaks were formed rather than discrete spots. The procedure employed for the crystallization of sucrose from the green coffee bean was unsuccessfully attempted on roasted coffee. However, paper chromatograms showed evidence for sucrose in the ethanol-water (95 to 5) eluent (clay column) of roasted coffee and crystallization was probably restricted by degradation products. Some hydrolysis of sucrose evidently occurred on roasting, because there was only weak evidence for glucose in the green coffee bean and no evidence for fructose. However, both glucose and fructose were identified by paper chromatography in the ethanol-water (95 to 5) eluent (clay column) of roasted coffee.

No attempt was made to determine the amount of chlorogenic acid found in this fraction quantitatively, as had been done for the green coffee bean.

Caffeine was not crystallized from roasted coffee bean, as it was from the green coffee bean, but was identified by paper chromatography using the new technique described previously.

The ethanol-water (80 to 20) fraction from the clay column contained the substances indicated in Table I. The paper chromatograms of the ethanol-water (50 to 50) fraction from the clay column showed no resolution, which indicated the possibility of polymeric materials. No free amino acids were found in the roasted coffee bean, which is in agreement with the work of Clements and Deatherage (*4*).

Table II is a compilation of the data on the fractionation of green and roasted coffee, leading to polysaccharide isolation. Before the polysaccharides of coffee can be separated, the low molecular weight materials must be removed. Some of these materials are easily oxidized and interfere with the holocellulose preparation. Although a clean-cut separation of one type of material from another is not accomplished, each of the fractionation procedures has a definite purpose, the total effect of which is to remove the low molecular weight materials.

Referring to Table II, the percentage of material soluble in ethanol-water (80 to 20) shows no significant difference for green and roasted coffee. The benzene-ethanol (2 to 1) extraction removes the lipides from the plant material. Table II shows a small decrease in the fat content of the roasted coffee as compared to green coffee. Much of this decrease is probably due to decomposition during the roasting process.

After removing the lipides, a water extraction at room temperature is possible. Underwood and Deatherage (*23, 24*) found that most of the protein (3%) which can be extracted from green Santos coffee beans is water-soluble. The water extract at 20° to 25° C. contained polysaccharide material in addition as evidenced by paper chromatography. The present water extractions did not completely remove the proteins, as the residue gave a positive ninhydrin test. The data of Table II show a decrease in water-soluble material from green coffee to roast coffee. Some of the water-soluble part of the green coffee undoubtedly underwent a change during the roasting process with some portions possibly volatilizing.

Dilute ammonium oxalate solution

removes the water-insoluble pectins of plants. There is little variation in the amount of apparent pectin in the green and roasted coffee. This extract probably contained trace amounts of pectic substances grossly contaminated with other polysaccharide materials.

The strong oxidizing ability of chlorine dioxide removes the lignin and leaves the carbohydrate material behind. The apparent lignin content—acidified sodium chlorite extract—rises from green to roasted coffee. This is probably due to decomposition of the carbohydrate material during roasting and extracting, thus making it soluble in the acidified sodium chlorite solution. An apparent lignin value of 2.4% was found in the green bean by the 72% sulfuric acid residue method (*16*). While some lignin may be present, it is certainly grossly contaminated by polysaccharide material.

The 10% potassium hydroxide–soluble portion constitutes the "hemicellulose" of the coffee. The increase in the hemicellulose portion of the roasted coffee is probably due to a change in the holocellulose portion during the roasting process.

Table II shows that there is a slight decrease in the 10% potassium hydroxide–insoluble holocellulose in roasted coffee as compared to green coffee. This indicates water-solubilization of the polysaccharide material by the roasting process. It is well established that pyrolysis of starches results in water-solubilization with the final production of 1,6-anhydro-β-D-glucopyranose (levoglucosan). Hudson and coworkers (*8*) report that the mannan of tagua palm seeds undergoes pyrolysis to form the water-soluble 1,6-anhydro-β-D-mannopyranose. It is probable that the holocellulose of green coffee undergoes some pyrolysis, with probable shifting (*22*) of linkages, during roasting, resulting in water-extractable materials. The increase in the acidified sodium chlorite and 10% potassium hydroxide extracts for roasted coffee, as compared to green coffee, probably arises from this decrease in the alkali-insoluble holocellulose fraction.

Because only 11.0% of the 10% potassium hydroxide–insoluble holocellulose of green coffee dissolved in cuprammonium hydroxide solution, and as this fraction was primarily mannose as shown by electrochromatography, it appears that there is very little, if any, cellulose in the green coffee bean.

Hydrolysis of the 10% potassium hydroxide–insoluble holocellulose of green coffee yielded D-glucose, D-galactose, D-mannose, and L-arabinose as indicated by paper chromatography and proved by the isolation of crystalline derivatives of each. The arabinose was readily split out by refluxing with 1.5% sulfuric acid. This result was not unexpected, because arabinose commonly occurs in nature in the labile furanose ring form.

Schulze (*17*) attempted to hydrolyze the carbohydrate component of coffee with 75% sulfuric acid, but a small residue of undissolved material always remained. As was indicated above, the authors encountered the same difficulty when attempts were made to hydrolyze the 10% potassium hydroxide–insoluble holocellulose of green coffee quantitatively by the 72% sulfuric acid treatment of Monier-Williams (*12*). Because the 10% potassium hydroxide–insoluble holocellulose of green coffee apparently did not completely hydrolyze in 72% sulfuric acid, it was decided to attempt the hydrolysis by using anhydrous sulfuric acid. Anhydrous sulfuric acid is known to be a powerful solvent for organic compounds and was applied to polysaccharides by Wolfrom and Montgomery (*29*). The technique used herein was to lyophilize the 10% potassium hydroxide–insoluble holocellulose to give better surface properties and then to dissolve the material in anhydrous sulfuric acid (hydrogen sulfate). After solution was complete, water was slowly added in the cold without effecting precipitation. The dilute acid solution was then heated to produce complete hydrolysis. As this method constituted a new procedure for hydrolyzing polysaccharides, it was decided to experiment with cellulose to determine if the method were feasible. Hydrolysis of cellulose by the procedure described in the experimental portion of this paper for the quantitative determination of the constituent sugars of 10% potassium hydroxide–insoluble holocellulose of green coffee resulted in a glucose yield of 90.3% of the theoretical as determined by the Somogyi reduction procedure (*21*). This result compared favorably with the yield of 90.7% recorded by Monier-Williams (*12*) for the hydrolysis of cellulose with 72% sulfuric acid. Thus, the anhydrous sulfuric acid appeared to be of comparable hydrolyzing ability to 72% sulfuric acid with the added advantage that it was a very powerful dissolving medium which would dissolve compounds not completely soluble in 72% sulfuric acid. The total recovery of 87.1% from the anhydrous sulfuric acid hydrolysis of the 10% potassium hydroxide–insoluble holocellulose of green coffee appears favorable, because of the difficulty of hydrolyzing the material. The value of 87.1% has been corrected for the arabinose loss occurring in the absolute sulfuric acid method by evaluating the arabinose released by mild acidity.

The value of 6.0% L-arabinose obtained by direct photometry of paper chromatograms compared favorably with the direct pentose value of 6.7% obtained by the authors and that reported by Schulze (*18*). This shows that all the pentose is L-arabinose and that it is easily acid-hydrolyzable. The use of 6.7% for arabinose makes no significant change in the ratios of the sugars reported above.

The value of 46.3% D-mannose obtained by precipitation as phenylhydrazone is slightly below the value of 48.5% obtained by direct photometry of paper chromatograms. This is probably due to the incomplete hydrolysis with the 72% sulfuric acid utilized in obtaining the former. The close agreement obtained for the L-arabinose and D-mannose values by direct methods and by paper chromatographic methods substantiates the use of direct photometry of paper chromatograms as a procedure for quantitative determinations of sugar mixtures and supports the values obtained for the sugars in the 10% potassium hydroxide–insoluble holocellulose of green coffee.

The chemical nature of the coffee polysaccharides is under further investigation.

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FLAVOR CHEMISTRY

Constituents of Meat Flavor: Beef

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The flavor precursors of cooked beef are water-soluble. Lyophilization of a water extract yields a powder concentrate that on heating develops a flavor similar to that of cooked beef. A standard technique for the heat treatment and fractionation of the flavor constituents in this powder is described. Small amounts of carbonyls, ammonia, and hydrogen sulfide have been found in the most volatile fraction. An oily, viscous, liquid mixture of very low vapor pressure, but with a strong aroma, has also been isolated.

ALTHOUGH AN UNDERSTANDING of the chemical composition of meat flavor is basic to many aspects of meat technology, an actual knowledge of meat flavor constituents is nearly nonexistent. Bouthilet (*1*, *2*) and Pippen and coworkers (*10*, *11*) studied the flavors of chicken broth and separated and identified several of the volatile constituents of chicken broth distillate. Classical studies of beef flavor chemistry (*9*) dealt largely with the analysis of beef extract, a product which is formed by the subsequent concentration of beef tissue hydrolyzates and is thus different from beef per se.

The present investigation was undertaken as part of a program on fundamental studies of meat composition. The flavor aspects of this program are expected to result eventually in identifying the naturally occurring substances which are responsible for flavor in beef, pork, and lamb, and in various products made from these meats. This paper deals with the methods used to fractionate fresh beef into potentially flavorful and nonflavored portions and with the preliminary chemical investigation of some of the isolated products.

A beef powder extract separated from raw beef has been heated under vacuum and the total volatiles have been condensed at low temperature. This condensate, in turn, has been analyzed for carbonyls, and the most volatile fraction has been examined for acidic and basic components.

Experimental

Freeze-Dried Beef Powder Extract. Fresh meat was aged for 10 days at 36° to 38° F. Several of the muscles were then dissected and stored at 0° F. As needed, 1.5 kg. of meat was thawed, fat and connective tissue were removed, and the trimmed beef was ground in an electric grinder maintained at 32° F. One part by weight of the ground beef was blended for 1 minute with 1.5 parts by volume of ice-cold, distilled water. The slurry was allowed to stand overnight at 32° F., blended again for 1 minute, and centrifuged at 4000 r.p.m. for 20 minutes in a refrigerated centrifuge kept at 28° F. The supernatant liquid was decanted, mixed with 1% w./v. of Filter-aid, and filtered under vacuum through a Büchner funnel. The filtrate was shell-frozen in a dry ice–isopropyl alcohol bath and lyophilized. The yield of dry powder was approximately 3.5% of the weight of the trimmed meat.

Distillation and Fractionation. A vacuum of better than 10^{-5} mm. of mercury was maintained by means of a two-stage oil diffusion pump backed by a mechanical vacuum pump. The fractionation train consisted of three fraction collectors. The vacuum system is shown schematically in Figure 1. Thirty to 35 grams of dried powder were placed in flask *a*. Vapor from a boiling liquid in flask *b* rose through the jacket, *c*, surrounding *a* and returned to *b* by the condenser. An auxiliary heating tape was wrapped around the jacket to ensure that vapor condensation did not take place around *a*. By the appropriate choice of liquid any desired temperature could be maintained.

In a given experiment, traces of moisture were first removed from the dried powder by evacuating the system ($<10^{-5}$ mm. of mercury) at room temperature for 4 hours. A Dewar flask containing liquid nitrogen was then placed around trap *d*. Maintaining the same vacuum, the solvent in *b* was brought to a boil and heating was continued for 6 hours. At the end of the heating period the vacuum stopcocks at *h* and *i* were turned off, the liquid nitrogen trap was removed from *d* and placed around trap *g*, and a dry ice–isopropyl alcohol freezing mixture placed around trap *e*.

Spontaneous distillation took place as the contents of trap *d* came to room