used in interpretation as these samples were immediately frozen and freeze-dried, while the figures of meat are from samples whose pretreatment prior to analysis is unknown.

Amino Acids. In general, none of the game meats tested differs significantly from beef, mutton, and pork in the levels of essential (and nonessential) amino acids; i.e., the nutritional value, as far as the protein is concerned, of any of these meats is at least as high.

The same cautionary remarks regarding pretreatment of samples prior to analysis must be applied when comparing these figures with those of beef, mutton, or pork. However, comparison within the group, elephant, kongoni, wildebeeste, zebra, and zebu, where the samples were treated exactly the same, is more valid. This shows that elephant and zebu cattle have higher content of essential amino acids than kongoni and wildebeeste.

In addition, lysine levels in elephant and zebu meat are unusually high. This may be an asset from the nutritional point of view, especially where lysine is comparatively low in the staple foods.

The level of available lysine in elephant meat, 9.6, is a higher value than anything previously encountered in meat protein.

Admittedly only one sample of each was analyzed, but the figures are suggestive that both elephant and zebu cattle are superior to the other ungulate animals and also may be superior to the domesticated animals in supplying essential amino acids. Further research is required to confirm the results.

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COFFEE CONSTITUENTS

Isolation and Characterization of Cellulose in the Coffee Bean

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The main polysaccharide of the green coffee bean is an insoluble, hard (1 → 4)-β-D-mannan. If this is removed by formic acid hydrolysis, there is left a glucan which is shown to be cellulose in an amount equal to 5% of the green coffee bean (dry basis). Proof for cellulose was solubility in cuprammonia, hydrolysis to glucose, and acetolysis to β-cellobiose octaacetate. The procedure for the last, as a test for cellulose, has been improved.

THE first publication from this laboratory on the carbohydrates of green coffee (*8*) reported that there was little, if any, cellulose present in the bean. The authors now wish to report the results of definitive experiments which prove conclusively the presence of cellulose in significant amounts. The previous failure to establish cellulose was based upon its apparent lack of cuprammonium solubility, due, it is believed, to interference by the encrusting β-D-(1 → 4)-mannan (*6*).

Crude Cellulose Fraction Isolation

As previously described (*8*), ground green coffee beans (Santos 4's) were extracted with ethanol-water (80 to 20). The insoluble residue was extracted successively at room temperature with benzene-ethanol (2 to 1) and thrice with water, then twice at 90° C. with 0.5% ammonium oxalate, to yield 48% of a gray residue. The holocellulose was prepared from this residue, essentially according to the modification by Whistler and associates (*4*) of the procedure of Wise and coworkers (*5*) by treatment with aqueous, acidified sodium chlorite (pH 4.5 to 5.0) under nitrogen at 75° C. for 1.5 hours; yield 42% (dry basis). This material, after extraction with 10% potassium hydroxide, was previously (*8*) reported to contain the following amounts of constituent sugars: D-glucose, 17.8%; D-mannose, 48.5%; D-galactose, 14.8%; L-arabinose, 6.0%, all as determined by acid hydrolysis and quantitative papergram densitometry.

An amount of 40 grams of this material (the holocellulose) was extracted for 4 days with hot formic acid (90%) in a Soxhlet extractor fitted with a coarse, sintered-glass funnel. The formic acid was removed from the residual "holocellulose" by evaporation under reduced pressure, followed by the addition and removal of water by distillation under reduced pressure. A colorless solid was obtained on freeze-drying; yield 9.0 grams (22.5%), ash (sulfate) <1%. Treatment of cotton linters in the same manner for 4 days resulted in a weight loss of ~20%.

Assay of Glucose in Formic Acid Residue

A 0.500-gram sample of the formic acid residue was added to 75 ml. of

sulfuric acid (melting point −8° to −10° C.) in a round-bottomed flask submerged in a sodium chloride–ice–water bath at −4° C. The flask was allowed to warm to 6° C. over a period of 8 hours when the amber-colored solution was essentially homogeneous. Under power stirring, the solution was frozen about the flask walls. Water was added slowly (while stirring) to the frozen material over several hours with continued cooling of the flask. The homogeneous solution was diluted to 3 liters to give a sulfuric acid concentration of approximately 3%. This solution was refluxed for 10 hours and neutralized with barium carbonate. The solids were removed by filtration, and the combined filtrate and washings were evaporated under reduced pressure to 1 liter. The filtration and concentration were repeated several times. The hydrolyzate was taken to a sirup under reduced pressure, refluxed with 100 ml. of methanol for 1 hour, and filtered. The combined filtrate and methanol washings were evaporated under reduced pressure to a sirup, which was dissolved in water, filtered through hardened filter paper, and diluted to 50 ml.

Quantities of this solution ranging from 0.010 to 0.075 ml. were applied from a microburet to paper chromatograms, and the sugars present were determined densitometrically as described previously (*8*): glucose, 39% (basis theoretical hydrolysis yield); mannose, trace. In another chromatogram, a periodate-permanganate spray indicator (*7*) revealed a spot with R_f twice that of glucose. The material in this spot is under further investigation.

α-Cellobiose Octaacetate

The procedure of Green (*1*) was modified. Two grams (6.5% moisture) of the above-described formic acid residue was kneaded into a mixture of acetic anhydride (8 ml.) and sulfuric acid (0.2 ml.), at ice-bath temperature, until completely wetted. The mixture was placed in an oven at 50° C. for 14 days. The resulting black solid was mixed with acetic acid (10 ml.) until the solids were suspended. The suspension was stirred with 500 ml. of cold water and transferred to a larger container with an additional 250 ml. of water. The whole was then stirred for 30 minutes. The solids were removed by filtration, washed free of acid with water, air-dried, and extracted with boiling ethanol (95%, 175 ml.). Crystals were obtained on cooling; yield 0.290 gram, melting point 220–221° C. Concentration of the mother liquor gave additional material; 0.095 gram, melting point 220° C. The above aqueous filtrate was extracted with chloroform (200 ml.). The chloroform extract was shaken with an aqueous solution of sodium bicarbonate and evaporated under reduced pressure to yield a solid. The ethanol-extracted residue was then extracted with chloroform (200 ml.), and the chloroform was removed to again yield a solid. The chloroform-extracted solids were combined, and upon solution in hot ethanol (95%, 75 ml.) produced crystals on cooling; yield 0.600 gram, melting point 220° C. Mother liquor concentration afforded an additional amount; 0.105 gram, melting point 217° C. The total combined crystalline material (1.09 grams, 26.5%) was recrystallized from ethanol (95%); yield 0.940 gram, melting point 223° C. unchanged on further crystallization or on admixture with authentic α-cellobiose octaacetate, $[\alpha]_D^{22}$ +40° (*c* 3.0, chloroform), x-ray powder diffraction pattern identical with an authentic specimen (*3*).

Control Experiment with Cotton Linters

These modifications were applied to the isolation of α-cellobiose octaacetate from cotton linters in an attempt to raise the 42% (highest) yield reported by Green (*1*). By following the above procedure, employing the chloroform extraction, a yield of 48.5% of α-cellobiose octaacetate was obtained from cotton linters.

Base Extraction of the Formic Acid Residue

The formic acid residue (10.0 grams) was stirred for 20 hours at 80° C., under nitrogen, with 30% potassium hydroxide (2.0 liters). The solids were removed by filtration, washed free of base, and dried; yield 5.40 grams. The 30% potassium hydroxide–insoluble material (hereinafter called holocellulose B) was hydrolyzed with absolute sulfuric acid in the same manner as previously described and was found to contain 82% glucose, and no other sugar. This fraction represents 5.1% of the dry weight of the green coffee bean. This material was subjected to acetolysis and failed to yield more α-cellobiose octaacetate (26.5%) than was earlier recorded.

Cuprammonium Hydroxide Solubility

Cuprammonium hydroxide was prepared according to the method of Launer and Wilson (*2*). To 20 ml. of the clear cuprammonium hydroxide was added cleaned copper wire, and 0.093 gram of holocellulose B. The stoppered mixture was shaken briefly. After 15 minutes, all of the material had dissolved and it was recoverable readily upon neutralization with hydrochloric acid.

When this experiment was repeated on the above-described formic acid residue, and with intermittent shaking for 1 day, complete solution was not obtained.

Results and Discussion

The nearly selective formic acid hydrolysis of the holocellulose of green coffee resulted in the isolation of a crude fraction that, on alkaline treatment, yielded a product giving 82% glucose upon hydrolysis, readily soluble in cuprammonia, and producing α-cellobiose octaacetate upon acetolysis. All of these criteria establish the presence of cellulose in the green coffee bean.

On the basis of the yields of α-cellobiose octaacetate and glucose, the amount of cellulose present in the green coffee bean is 5.1% (dry basis). This work in no way alters the glucose content of the bean as previously reported (*8*) but establishes that at least 5.1% of the 7.5% glucose found is present as the β-D-(1 → 4)-glucan.

The initial hydrolysis was effected by nearly anhydrous sulfuric acid, a method described previously (*8*), except that better solution of the polysaccharide was obtained if the acid was adjusted, by its melting point, to slightly on the fuming (excess sulfur trioxide) side.

The procedure for cellulose acetolysis, described by Green (*1*), was improved by adding a chloroform extraction as ethanol alone does not extract all of the product from the crude acetolyzate. Applied to cotton linters, a yield of 48.5% of α-cellobiose octaacetate was obtained as compared with the highest value of 42% reported by Green (*1*). When this improved method was employed, a yield of 26.5% of α-cellobiose octaacetate was obtained from the cellulose preparation from green coffee. One may conclude that the previous treatment with hot formic acid followed by hot alkali had altered the polysaccharide so that it gave a lower yield of disaccharide, although affording a good yield (82%) of glucose upon hydrolysis.

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