samples in the study. Under any chromatographic conditions we investigated, similar results were obtained, i.e., the glycoalkaloids in both samples had identical R_f values, and only by the use of $SbCl_3$ as spray reagent was it possible to detect that different glycoalkaloids were present in the samples, by the intensity of violet color development. GLC traces of the hydrolyzates of R_f 0.25 from both samples are shown in Figure 2b. One sample was composed of demissidine glycoalkaloids almost exclusively. The other sample was a mixture of demissidine and solanidine glycoalkaloids.

Anomalous peaks that were found in the GLC analysis in most cases appeared to occur because of lack of complete removal of lipids in the initial isolation of the glycoalkaloid fraction; these peaks did not interfere with the analysis but could be removed by washing the glycoalkaloid precipitate with chloroform.

Although we investigated only glycoalkaloids containing the aglycones solanidine and demissidine, the method should also be applicable to such pairs as tomatidine (Δ5 saturated) and tomatid-5-en-3-ol (Δ5 unsaturated).

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Received for review November 24, 1976. Accepted January 26, 1977. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Polyamines in Green and Roasted Coffee

Three polyamines, putrescine (1,4-diaminobutane), spermine (α,δ-bis(γ-aminopropylamino)butane), and spermidine (α-(γ-aminopropylamino)-δ-aminobutane), have been isolated from green coffee beans and identified by thin-layer chromatography. During the roasting process these polyamines are degraded, probably being precursors of coffee flavor formation. However, coffee beans of the same variety and harvested in the same year, which produced different beverage quality, showed similar polyamine content. No nitrite could be detected in the green bean, which would avoid nitrosamine formation during the roasting process.

Polyamines are widely distributed in plants (Smith, 1971), and they have also been found in many seeds (Moruzzi and Caldarera, 1964). Recently, Wang and his co-workers (Wang et al., 1975) showed that putrescine, spermine, and spermidine have a threshold level (50% correct response) between 10^{-4} and 10^{-5} M in water. The odor of these polyamines in water is a putrid one, but if polyamines form salts with glutamic acid, the flavor of soy sauce could be improved (Udo, 1931, 1932).

The price of coffee beans depends on the flavor of the infusion of the roasted beans, and it would be of interest to know if polyamines exist in green coffee, their behavior during the roasting process, and if coffees with different qualities of beverage within the same variety have the same polyamine content.

MATERIALS AND METHODS

Commercial coffee beans (*Coffea arabica* L. var. Mundo Novo) were harvested in 1974 from different regions of the State of São Paulo and Minas Gerias, Brazil. After roasting and brewing, they were classified by professional tasters with regard to the quality of the beverage as either Soft (mild flavor) or Rio (strong medicinal flavor). Samples not well characterized were discarded.

The beans (500 g) were roasted in a commercial roaster at 240 °C for 12 min. One sample was taken at 9–10 min, which represents the light roast (9–10% weight loss), and the other at 12 min, which represents the dark roast (15–17% weight loss).

Polyamine Determination. Coffee beans were ground in a Microbroyeur Quantitatif Dangoumau (Prolabo, Paris, No. 7499.02), and 5 g of the powder (10 g for the roasted) was immediately extracted with 60 mL of 5% trichloroacetic acid by shaking for 2 h. After centrifugation, the total volume and the supernatant were measured, and the residue was discarded. Successive extractions with 5% Cl_3CCOOH proved unnecessary if the volume retained by the residue was taken into account for correction.

Extraction with 75% ethanol acidified with 4 N HCl to pH 4.0 removed the same amount of putrescine as 5% Cl_3CCOOH, but the recovery of spermine and spermidine was much smaller. Ethanol without acidification extracted less putrescine than 5% Cl_3CCOOH.

After preliminary purification of the supernatant with Dowex-50 W-X8 (H^+) resin (20–50 mesh) according to Smith (1970), the amines were dansylated and chromatographed using 250-μm thick Kieselgel G (Merck) commercial TLC plates and cyclohexane/ethyl acetate (3:2 and 1:1) as solvent.

Putrescine, spermine, and spermidine were estimated by fluorometry of their dansyl derivatives in TLC plates using a Vitration TLD 100 densitometer (Smith, 1973). Two replicates were made for each coffee sample.

The identity of the polyamines was checked by cochromatography and by comparing with pure compounds in the following solvent systems: cyclohexane/ethyl acetate (3:2), cyclohexane/diethyl ether (1:9), and cyclohexane/chloroform (1:19) by using the same TLC plates mentioned herein.

The following R_a (relative to ammonia) values were recorded for the dansyl derivatives: putrescine, 0.81; spermidine, 0.60; and spermine, 0.44 (solvent system,

Table I. Polyamine Content in Arabica Green and Roasted Coffee Beans Which Differ in Beverage Quality (Dry Weight Basis)

Coffee sample	Green			Roasted,
	Putrescine, μg/g	Spermidine, μg/g	Spermine, μg/g	putrescine, μg/g
Soft 1	54	20	10	2
Soft 2	42	16	8	2
Soft 3	38	15	8	1
Soft 4	40	14	7	2
Rio 1	44	15	9	3
Rio 2	43	15	7	2
Rio 3	37	15	8	2
Rio 4	43	15	8	2

cyclohexane:ethyl acetate, 3:2).

Nitrate and Nitrite Determination. Green coffee beans were ground, and 1 g of the powder was extracted with 25 mL of 1% potassium aluminum sulfate by shaking for 1 h. After centrifugation, the supernatant was subjected to nitrate analysis by means of a specific electrode (Nielsen and Hansen, 1976) and nitrite according to the colorimetric method of Adriaanse and Roberts (1969).

RESULTS AND DISCUSSION

The polyamines putrescine, spermine, and spermidine were identified by thin-layer chromatography through their dansyl derivatives. This seems to be the first report of these polyamines in coffee (Vitzthum, 1975). Table I shows the results of polyamine estimation in green and light roasted coffee beans. No polyamines could be detected in the dark roast; only putrescine could be detected in the light roasted coffee.

Green coffee of the same variety, harvested in the same year, and having different organoleptic properties shows approximately the same amount of putrescine, spermidine, and spermine.

It seems that the polyamine content has no connection with the Rio flavor; however, it is well known that the amino group is important in the formation of coffee taste and aroma (Baltes, 1975) through different types of reactions. Putrescine can be converted into pyrrolidine by heating (Lijinsky and Epstein, 1970) during roasting. Cadaverine and piperidine were found in the basic fraction of flavor components in raw soybean (Arai et al., 1966). If the polyamines herein studied are degraded in the roasting process, it is possible that they contribute to the coffee flavor, in a general manner.

It is well known that polyamines are precursors of carcinogenic nitrosamines (Lijinsky and Epstein, 1970; Bills et al., 1973). Although nitrate is present in commercial coffee beans (620 mg/100 g, this paper), no nitrite could be found in these samples (sensitivity of the method ≃75 μg/100 g). The appreciable amount of ascorbic acid in coffee beans (25–60 mg/100 g) (Vasudeva and Gopal, 1974) may contribute to inhibition of nitrosamine formation as postulated by Gray and Dugan (1975) in model food systems.

ACKNOWLEDGMENT

The authors thank Claudio Berreta for technical assistance.

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Received for review April 30, 1976. Accepted December 27, 1976. Research was supported by grants from the Brazilian Coffee Institute.