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RIBONUCLEIC ACID FROM COFFEE BEANS

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Key Word Index—*Coffea arabica*; *Coffea robusta*; Rubiaceae; extraction medium; RNA; spectral properties; nucleotide composition; hyperchromicity.

Abstract—A method of extraction of RNA from coffee based on phenol treatment is described. Effects of various agents and pH of the extracting buffer on the efficiency of extraction were studied. The best extracting solution is 0·2 M Tris-HCl buffer at pH 7·4 with 1% sodium dodecyl sulphate and 0·05% EDTA. RNA (5–6%) is lost in the tissue residue and 4·6% in the interphase layer. No significant deviation of the spectral characteristics of the RNA solutions obtained from three samples of coffee from that for purified yeast RNA is observed. The purine–pyrimidine ratio for the RNA has been found to be in the range of 1·25–1·38.

INTRODUCTION

The presence of caffeine in high amount in coffee bean[1] is well-known. The processing of coffee beans, which imparts a delicious flavour to coffee, brings about an increase in the caffeine content.[2] An investigation into the total ribonucleic acid content of coffee bean has therefore been made in view of the earlier work[3] reported from this laboratory on RNA content of tea leaves.

By excluding interfering effects of chlorophyll, other pigments and tannin materials, Vanyushin and Belozersky[4] analysed the total RNA of seed, pollen and thallus material in a systematic and extensive study. Trim *et al.*[5] extended the work in analysing leaves of wheat, sugar-beet and potato. Though the RNA content of tea in its various stages of processing[3] has been recently studied, similar work on coffee beans is still lacking. In our paper investigations are reported on the changes the processing of coffee bean brings about upon the RNA as revealed by base composition spectral characteristics and the secondary structure of RNA isolated from coffee in various stages.

RESULTS

A marked decrease in RNA content during the processes of curing and roasting has been observed, the decrease being more or less gradual from fresh berry to roasted beans. Of the two varieties of coffee tested, it is evident from Table 1 that Arabica coffee has a higher RNA content than the Robusta type.

Isolation of RNA from Coffee Bean

In the course of isolation of RNA from coffee beans by Kirby's method,[6] the extracting medium and pH were shown to be of great importance (Table 2). In spite of efficient

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[6] K. S. Kirby, *Biochem. J.* **66,** 495 (1957).

extraction by 1·7 M sodium chloride at 100°, the use of this extracting method was not given prior importance in view of partial degradation of the extracted RNA. Severe green discoloration which occurred when using 0·2 M Tris-HCl at pH 8·0 discouraged the use of this medium. Since the same percentage extraction of RNA was observed with 0·2 M Tris-HCl buffer at pH 7·4, without serious discoloration, this medium was preferred. Addition of other chemicals to Tris-HCl buffer can improve RNA extraction (Table 2). As bentonite produced a brown coloured mass, it was considered to be inferior to 1% sodium dodecyl sulphate plus 0·05% EDTA.

Table 1. Changes of RNA content during processing of coffee beans

Variety of coffee	State of coffee	Protein content (% fresh wt)	RNA content (μg/mg protein)
Arabica Plantation PB	Fresh berry	16·25 ± 0·5	120 ± 1·0
	Green bean	17·5 ± 0·2	80 ± 0·8
	Roasted bean	19·3 ± 0·2	35 ± 0·7
Robusta Plantation PB	Fresh berry	15·2 ± 0·4	110 ± 0·7
	Green bean	16·8 ± 0·3	75 ± 0·8
	Roasted bean	18·3 ± 0·4	25 ± 0·8

The results are given as mean ±s.e. of the mean.

In our system, therefore, the best homogenizing medium for the extraction of minimally degraded RNA is one which consists of 0·2 M Tris-HCl buffer of pH 7·4, 1% sodium dodecyl sulphate and 0·05% EDTA.

Table 2. The effect of different extractants on extraction of RNA from fresh berry, green bean and roasted seeds of coffee

Extractant	% Extraction of RNA from: Fresh berry		Green bean		Roasted seed	
Phosphate buffer (0·1 M)						
pH 6·8	37		28		30	
pH 8·0	56		52		54	
Sodium chloride						
0·1 M, 2°	62		60		61	
1·7 M, 2°	65		57		58	
1·7 M, 100°	78		78		77	
Tris-HCl (0·2 M)						
pH 8·4		65				
pH 8·0	72	78	74	77	72	78
pH 7·0		67		67		68
pH 6·0		64		62		63
pH 7·4, alone	73	77	73	78	73	78
+ 1% bentonite		85		85		84
+ 1% sodium dodecyl sulphate		79		79		79
+ 1% sodium dodecyl sulphate + 0·05% EDTA		86		86		87

Properties of the RNA Isolated from Coffee

Spectral characteristics, nucleotide composition and the secondary structure, in terms of hyperchromicity due to the action of alkali, heat and hydrolysis with pancreatic ribonuclease, of RNA isolated from coffee are given in Table 3.

TABLE 3. SPECTRAL CHARACTERISTICS, NUCLEOTIDE COMPOSITION AND OPTICAL PROPERTIES OF TOTAL RNA ISOLATED FROM FRESH BERRY, GREEN BEAN AND ROASTED SEEDS OF COFFEE

Property	Fresh berry	Green bean	Roasted seed
UV absorption (nm)			
A_{max}	261	261	260
A_{min}	231	232	232
Absorbance ratios			
A_{max}/A_{280}	1·64	1·63	1·62
A_{max}/A_{min}	1·78	1·80	1·78
Nucleotide composition (mole %)			
AMP	31·6 ± 0·2	30·8 ± 0·4	27·4 ± 0·2
GMP	31·6 ± 0·4	31·4 ± 0·2	32·1 ± 0·3
CMP	25·7 ± 0·2	25·6 ± 0·3	24·2 ± 0·6
UMP	20·9 ± 0·2	21·2 ± 0·3	23·2 ± 0·4
Purine/pyrimidine ratio	1·38	1·32	1·25
(AMP + CMP)/(GMP + UMP) ratio	1·16	1·1	0·97
(AMP + UMP)/(GMP + CMP) ratio	0·93	0·92	0·88
Hyperchromicity (%)			
Increase in A_{260} on alkaline hydrolysis*	33	32·7	32·8
Increase in A_{260} on heating†	20·8	20·5	20·2
Increase in A_{260} on enzymic hydrolysis‡	28	27·3	27·1

Duplicate determinations on each of three preparations for each stage of coffee were made. A diluted solution of RNA (*ca.* 15 μg/ml) was used for spectral measurements.

* 0·3 N KOH for 18 hr at 37°.

† From 25–95° according to the method of Montagnier and Bellamy.[7]

‡ With pancreatic ribonuclease according to Anfinsen *et al.*[8]

RNA isolated from fresh berry, green and roasted beans of coffee possesses spectral characteristics very similar to those reported by other workers for highly polymerized RNA from other sources.[9] The nucleotide composition data show that the RNA preparations from all the sources are characterized by a high purine-pyrimidine ratio. Through the processing steps, the ratio decreases. The 6-amino/6-keto ratio also decreases. The unique processes of curing and roasting does not bring about any significant change in the hyperchromicity value, thereby establishing the heterogenity of the RNA molecule obtained from coffee samples.

UV absorption by solutions of RNA from coffee increases gradually with rise in temperature, as other workers have also described.[10] On cooling to 25°, absorbance falls to its

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[8] C. B. ANFINSEN, A. R. RADFIELD, W. L. CHOATE, J. PAGE and W. R. CARROLL, *J. Biol. Chem.* **207,** 201 (1964).

[9] P. DOTY and R. HASELKORN, *Ann. N.Y. Acad. Sci.* **81,** 693 (1959).

[10] A. J. MUNRO, *Biochem. J.* **91,** 210 (1964).

previous value, indicating that the bonds are intra- rather than inter-molecular. Since the transition in absorbance is gradual, the exact melting temperature is difficult to determine. Therefore, the melting temperature (T_m) has been defined as that at which 50% of maximal absorption is achieved. For fresh berry RNA, it is 65°, which is lower than those for green bean (65·5°) and roasted bean (66°). The similarity in melting curve and closeness of T_m for fresh berry, green bean and roasted seeds of coffee are in good agreement with the similarity of nucleotide composition of RNA from the three sources. The purified RNA isolated from fresh berry is more rapidly digested by crystalline pancreatic RNase than RNA samples from green and roasted coffee beans.

DISCUSSION

The coffee berry, perishable in the raw state, requires curing and roasting. The progressive decrease in RNA content from fresh berry to roasted seed (Table 1) seems to be due to chemical or enzymatic breakdown of macromolecular RNA constituents during processing.

In applying Kirby's method of RNA isolation to coffee beans, care has been taken to isolate the product in a minimally degraded state. A major difficulty encountered is that coffee bean homogenates in Tris-HCl buffer assume a light brown, then light green and finally a green colour. The colour deepens markedly as the pH of the extracting buffer is raised above 7·4. Northmore,[11] investigating the problem in Kenya coffee, concluded that chlorogenic acid, usual constituent of coffee bean, produces this characteristic colour on complexing with magnesium. Hence the use of EDTA during the isolation of RNA is highly recommended. Cacao bean has been found to contain an appreciable amount of copper,[12] which may form complexes with RNA through guanine.[13] A greenish blue colour is therefore not unlikely. However, this possibility is ruled out by the very low copper content of coffee beans. The presence of coffee RNase and excess of interfering UV-absorbing substances also pose problems. Satisfactory isolation has been achieved by the use of 0·2 M Tris-HCl buffer of pH 7·4 in presence of 1% sodium dodecyl sulphate and 0·05% EDTA and by repeated precipitation of RNA in 95% ethanol and dissolution of the precipitate in Tris-HCl buffer at pH 7·0.

In the modified Kirby's method applied to coffee beans, 4–5% of the total RNA is lost in the phenol–water interphase during phenol treatment. This fraction of RNA has not been investigated, although phenol-soluble RNA fractions have been reported.[14] Despite repeated precipitation and dissolution, the purified RNA solution from coffee beans contains nearly 0·9% DNA and about 4·6% protein.

The purified RNA samples from coffee beans have purine/pyrimidine ratio in the range 1·25–1·38, values well above that for tobacco leaves or sugar-beat leaves[15] having high cell-wall residues. However, these high values of purine/pyrimidine ratio in coffee bean RNA may be related directly or indirectly to the occurrence of different types of free purine bases.[1,16]

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No significant variations in optical properties of RNA from the three stages of coffee have been observed. This may be correlated with the view that the secondary structure of RNA as isolated from coffee is not greatly altered during the various stages of processing.

Our studies are continuing.

EXPERIMENTAL

Fresh coffee berries. were a gift from the Coffee Experimental Research Station at Balehnnur, Mysore, India. They were fermented in the sun at daytime and under cover at night for 2 or 3 weeks. When fermentation was complete, the dried berries were pounded whereby the pulpy coverings were removed to expose the cured or *green beans.* The green beans were roasted at 200° in a rotating cylinder. The end of roasting was indicated by the appearance of oil on the surface of the bean. The colour changed from original raw to almost black. The *roasted beans* were then subjected to quick cooling.

Estimation of ribonucleic acid content. 200 mg samples of coffee beans were homogenized in ice-cold 80% EtOH, centrifuged, and the supernatant discarded. The insoluble material was washed in the following sequence of 5 ml vol. of solution: twice in cold EtOH, thrice in EtOH–Et_2O-$CHCl_3$ (2:2:1) for 15 min at 35° until all pigments were removed and finally twice in 0·2 M perchloric acid. Except for EtOH Et_2O $CHCl_3$, the washing was for *ca.* 5 min at 0–4°. The washed precipitate was then extracted with 0·5 M perchloric acid for 20 min at 90° with constant slow shaking. The RNA content of this acid extract was determined by the orcinol colour method of Mejbaum.[17]

Isolation of RNA. 40 g samples of ground coffee beans were homogenized in a chilled mortar with 100 ml of cold 0·2 M Tris-HCl buffer, pH 7·4, containing 0·05% EDTA and 1% sodium dodecyl sulphate. The homogenates were then squeezed through cheese cloth. The filtrate was transferred to a glass-stoppered bottle and stirred for 2 hr at room temp. with an equal vol. of H_2O-saturated re-distilled phenol. Centrifugation at 1000 *g* for 30 min at 0° resulted in the separation of an aqueous phase with an interphase layer, a phenol phase and a slight sediment at the bottom of the tube. The upper aqueous layer was removed and the phenol phase (interphase) was washed wtih an equal volume of Tris-HCl buffer. The mixture was centrifuged in the cold for 15 min and the aqueous wash phase was pipetted out and mixed with the previous aqueous portion. RNA was precipitated by adding 2·2 vol. of 95% EtOH containing 2% potassium acetate to the chilled aqueous extract and the mixture was kept at −10° overnight. The RNA precipitate was re-removed by centrifugation at 3000 *g* at 0° for 20 min and then dissolved in 0·2 M Tris-HCl pH 7·0. The deep brownish solution was brought to pH 4·5 (1 N HCl) at which pH part of the interfering colours disappeared. The precipitation centrifugation and dissolution steps were repeated several times. RNA was finally purified by dialysing for 24 hr at 0–4° against three changes of glass-distilled water. The diaylsed solution was clarified by centrifugation at 10 000 *g* for 20 min. at 0° and the clear supernatant was used for determination of spectral and other characteristics.

Determination of nucleotide composition. RNA was hydrolysed with 0·3 M KOH solution at 37° for 18 hr according to the modified procedure of Schmidt and Thanhanser.[18] Unhydrolysed material was removed by bringing the solution to pH 2·0 (5 N perchloric acid) and centrifugation in the cold. The supernatant was neutralized (2 N KOH) and potassium perchlorate crystals removed by centrifugation in the cold. The supernatant, contained the products of hydrolysed RNA in the mononucleotide form, was partially concentrated *in vacuo* over calcium chloride and mononucleotides were separated by PC using *iso*-butyric acid–0·5 N ammonia (5:3) solvent system according to Magasanik *et al.*[19] After development (18 hr), the sheet was air-dried (1 hr) and then oven-dried (85°, 20 min). The positions of the nucleotides were marked lightly by pencil under UV. They were extracted by dipping the marked centres of nucleotides in exactly 4 ml of 0·067 M phosphate buffer (pH 7·1) at 37° for 18 hr. The amount of each nucleotide was calculated by the absorption measurement of the extracted nucleotides according to the method of Elson, Gustafson and Chargaff.[20]

Protein was estimated by first estimating nitrogen of coffee beans by the Micro-Kjeldahl method of Ma & Zuazaga,[21] and multiplying by 6·25.

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