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SHORT COMMUNICATION

ALBUMIN FRACTIONATION OF GREEN COFFEE SEED VARIETIES BY ACRYLAMIDE GEL-ELECTROPHORESIS

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Abstract—Approximately a dozen protein fractions have been found in aqueous extracts of green coffee seeds by means of disk-electrophoresis on polyacrylamide gel. The best separation was obtained when 6 M urea was present in the gel. The albumin extracts obtained from different varieties of *Coffea arabica* and of *C. canephora* exhibit the same general electrophoretic pattern.

INTRODUCTION

MANY authors have studied the chemical composition of green coffee seeds,[1] but their protein content has not been extensively investigated,[2–5] despite the fact that the albumins amount to about 2·5 per cent of the seeds dry weight.[6] This report deals with the methods for characterization of these albumins by disk-electrophoresis on polyacrylamide gel. Furthermore the electrophoretic patterns of albumins extracted from four varieties of *Coffea arabica* (about 90 per cent of world production) and from two varieties of *C. canephora* (9 per cent of world production) are compared.

RESULTS

In Fig. 1, the electrophoretic patterns of albumin extracts of green coffee seeds obtained by disk-electrophoresis on polyacrylamide gel in a glycine–tris buffer system (pH 9·5) and in glycine–acetic acid buffer system, containing urea (pH 2·3), are shown. As can be seen, the patterns obtained at the two pHs are very different. The alkaline buffer system reveals three fractions (M_b 0·13, 0·26, 0·80) which represent together approximately 85 per cent of the total protein content, while the acid buffer shows a more uniform distribution of the protein content between eleven fractions (M_b 0·11, 0·14, 0·24, 0·30, 0·34, 0·46, 0·49, 0·55, 0·64, 0·68 and 0·77). The reproducibility is excellent for identical samples under identical conditions whether analysed contemporaneously or not. No improvement of the fractionation in the alkaline buffer could be obtained by dilution of the protein extract which would indicate that

[1] M. SIVETZ, in *Coffee Processing Technology* (edited by the Avi Publishing Company, Inc.), p. 165, Mack Printing Company, Easton, Pennsylvania (1963).
[2] G. E. UNDERWOOD and F. E. DEATHERAGE, *Food Res.* **17**, 425 (1952).
[3] C. P. NATARAJAN, *Bull. Centr. Food Technol. Res. Inst.* **4**, 260 (1955).
[4] L. E. BARBERA, *Coffee Tea Inds.* **79**, 12 (1956).
[5] R. L. CLEMENTS and F. E. DEATHERAGE, *Food Res.* **22**, 222 (1957).
[6] A. BOMER, A. JUCKENALK and J. TILLMANS, in *Handbuch der Lebensmittelchemie*, p. 9, Verlag Von Julius Springer, Berlin (1934).

the albumin band of M_b 0·13 is not produced by an overlapping of several bands of a similar mobility caused by overloading.

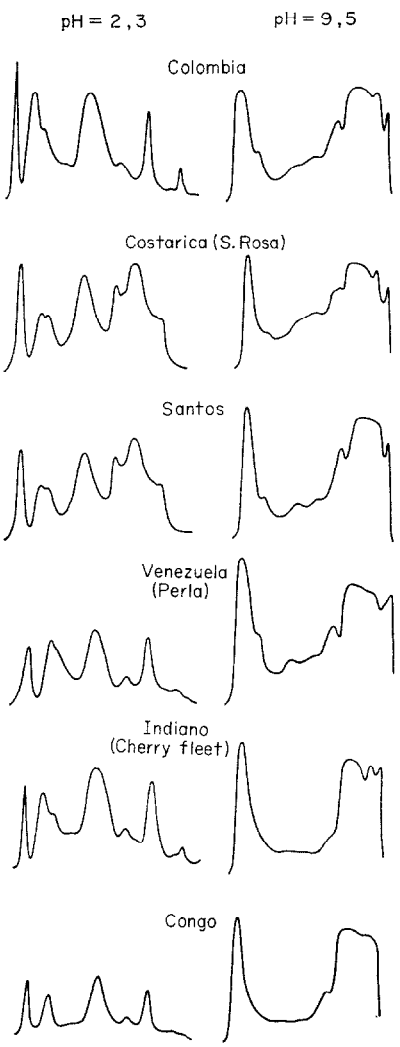

FIG. 1. COMPARISON OF DISK-ELECTROPHORETIC PATTERNS OF DIFFERENT VARIETIES OF *Coffea arabica* AND OF *Coffea canephora*.

Profiles show protein separations from right to left at pHs 2·3 and 9·5 respectively. Key: Colombia, Costarica, Santos, Venezuela are varieties of *C. arabica*; Indiano and Congo are varieties of *C. canephora*.

In the acid buffer system, urea must be present in the gel. In fact, on adjusting the pH of the aqueous extracts of green coffee seeds to 2·3 with 2 N HCl, the formation of a flocculent brown protein precipitate was observed, whereas when 6 M urea was present, this did not occur. The substitution of ammonium persulphate by riboflavin,[7] when urea is present, does not substantially change the albumin pattern.

[7] J. M. BREWER, *Science* **156**, 265 (1967).

As can be seen in the figure, different coffee varieties have a similar general pattern at both pHs, but also show some small varietal differences.

EXPERIMENTAL

Materials

Four varieties of *Coffee arabica* (Columbia-Armenia; Costarica-S. Rosa; Brasile-Santos; Venezuela-Perla) and two of *C. canephora* (Indiano-Cherry Fleet; Congo) were used.

Preparation of Green Coffee Seeds Extracts

Dry whole seeds of each green coffee sample were finely crushed and 10 g of the powder were dispersed in 50 ml of water by mild mechanical stirring for 3 hr at room temperature. Clear extracts were obtained by centrifuging the mixture at 30,000 × *g* for 20 min.

Apparatus and Electrophoretic Method

Disk-electrophoretic runs were carried out with freshly prepared albumin fractions according to Ornstein[8] and Davis[9] in a vertical apparatus of Canal Industrial Corporation, Rockville, Maryland. The acrylamide concentration was 7·5% (w/v); the solutions for gel and buffer preparations are similar to those described in the gel formulation sheets (Shandon Scientific Co., U.K.) for a gel system at pH 9·5 and pH 2·3. The urea solutions used for gel preparation were free of cyanate.[10] For gel preparation vertical glass columns 6·5 × 0·5 cm were used. 80-μl samples of albumin solutions in 11% (w/v) sucrose were loaded on the top of the gel, with bromophenol blue and safranine respectively as tracking dyes; 2·5 mA per channel were applied for 20 min at pH 2·3. The gels were then stained overnight with 5‰ (w/v) aniline blue-black in 7·5% (v/v) acetic acid. The excess of stain was removed by electrophoretic destaining in 7·5% (v/v) acetic acid, applying 12·5 mA per channel and the intensity of the protein bands was measured with a microdensitometer Chromoscan (Joyce, Loebl & Co. Ltd., Gateshead, England). The mobility of protein bands is referred to that of bromophenol blue (at pH 9·5) and of safranine (at pH 2·3) taken as 1.

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[8] L. Ornstein, *Ann. N.Y. Acad. Sci.* **121**, 320 (1964).
[9] B. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
[10] E. G. Cole and O. K. Mecham, *Anal. Biochem.* **14**, 215 (1966).