A PAPYROGRAPHIC STUDY OF THE NONPROTEIN NITROGEN OF MANGOES (*MANGIFERA INDICA LINN.*)

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PARTITION chromatography on filter paper, '*Papyrography*'[1,2] offers an elegant, simple and rapid method for a study of the content of free amino acids and simple peptides in mixtures and in extracts. Dent, Stepka and Steward[3] pioneered the use of this method to the study of plant tissue extracts. Joslyn and Stepka[4] recently reported what is perhaps the only study of free amino acids in some fruit pulps. We have carried out a papyrographic study of the non-protein nitrogen fraction of the four common varieties of mango (*Mangifera indica*).

The rind free pulp of the four varieties of mangoes, Malgova, Raspuri, Alfonso and the ungrafted country variety, was extracted with acidulated alcohol (final alcoholic concentration in the mince reaching 80 per cent. by

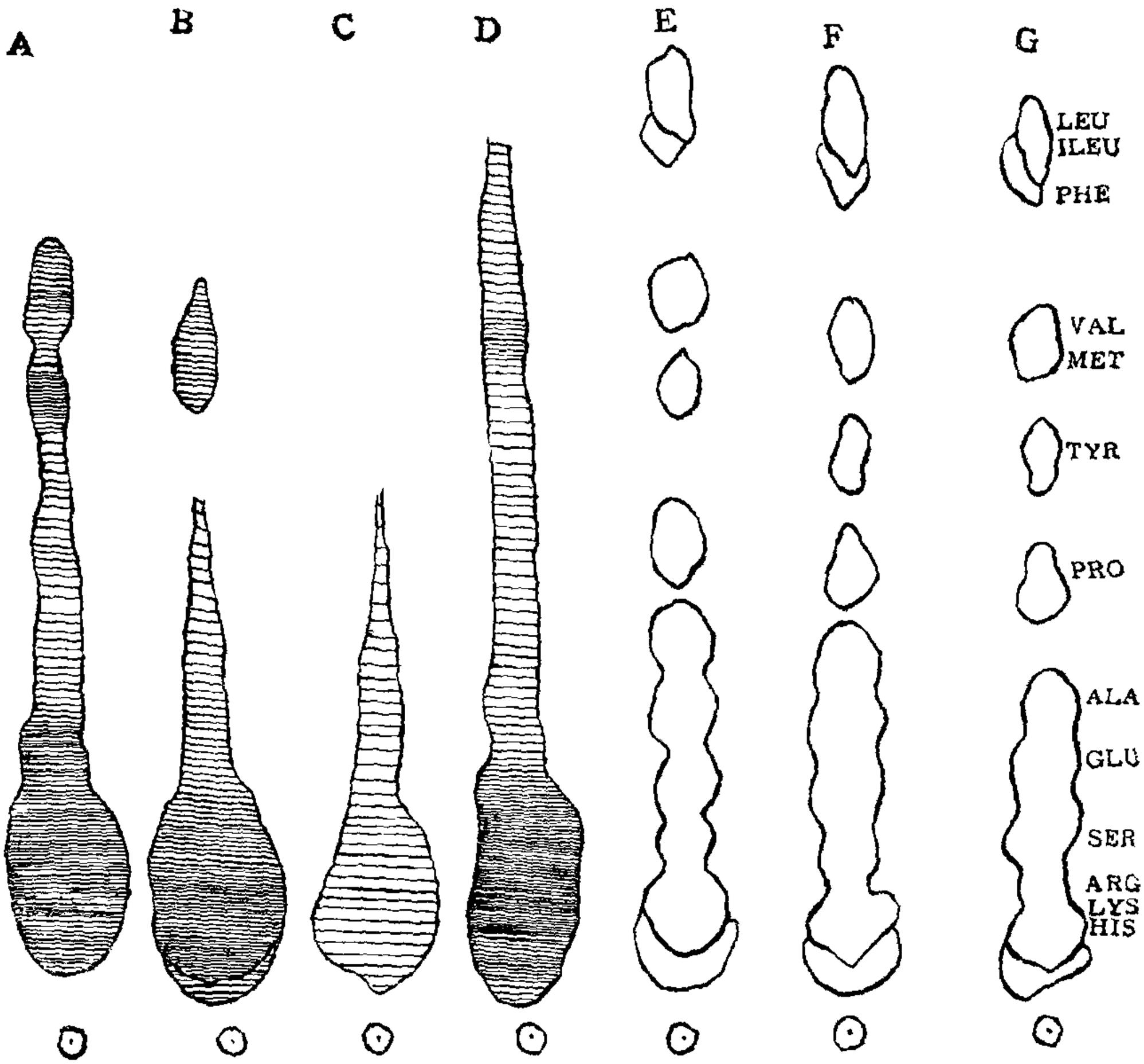

FIG. 1

A—D: Chromatograms of alcoholic extracts of four varieties of mangoes. A: Ungrafted variety; B: Raspuri; C: Alfonso; D: Malgova. Note:—Relative intensities of colour of spots indicated by shading. Names of amino acids abbreviated according to Brand and Edsall, *Ann. Rev. Biochem.*, 1947, **16**, 223. FIG. 1 E Chromatogram of a sample of Casein hydrolysate (acid). FIG. 1 F: Chromatogram of Casein hydrolysate (acid) with 10 per cent glucose. FIG. 1 G: Chromatogram of Casein hydrolysate (acid) with 20 per cent. glucose.

volume), in a Waring blendor.[4] The extracts were concentrated to a syrup in a desiccator under vacuum over concentrated sulphuric acid. It was taken up with 1/5 the volume of water, which leaves most of the carotenoid pigments undissolved. The aqueous solution was extracted with ether to remove traces of the remaining pigments. The total nitrogen-content of these extracts was determined by direct Nesslerisation after digestion and equalized.

The concentrated extracts were chromatographed in one dimension at a level of 10×10^{-3} ml. by the ascending technique and with *n*-butanol-acetic acid as the developing solvent, as described earlier.[5] Ordinary diet jars with ground glass plate covers and Whatmann No. 1 paper 20 cm. × 20 cm. were used so that the four samples and reference mixtures could be chromatographed simultaneously.

Obvious conclusions from the chromatograms (see Fig. 1) are, (1) that the order of richness of ninhydrin-positive substances is: Mulgova, Raspuri, ungrafted variety, Alfonso being least; (2) that except for some weak spots which reveal the presence of some of the usual amino acids, all the extracts yield a continuous, big, pear-shaped and characteristic spot below the position usually occupied by glutamic acid. Though Joslyn and Stepka report that "peptides do not, as a rule, occur in detectable amounts in the cold alcohol extracts of plant tissues", it is not possible to explain such a heavy concentration of ninhydrin-positive substances (see Fig 1) except by assuming the presence of peptides.

The extracts contain high percentages of reducing sugars (up to 20 per cent.) which, it was thought, might interfere with the chromatographic picture. Casein hydrolysates enriched with 10 and 20 per cent. of glucose were prepared and chromatographed simultaneously with sugar-free casein-hydrolysate. In presence of the sugar, a slight decrease of the R_F values was observed, but the general clarity of the picture, the relative positions of the amino acids and their separation were not affected. The reducing sugar was then removed as the osazone and after removal of the excess of phenylhydrazine from the mother-liquor by extraction with ether, the casein-hydrolysate was chromatographed. This step did not result in any detectable alteration in the picture, showing thereby, that the presence of sugar does not materially affect the separations.

With a view to elucidate the nature of the peptides suspected to be associated with the extracts, the samples were hydrolysed in a sealed tube with 6N HCl at 100° C. for 24 hours. The removal of the acid from, and the concentration of, the hydrolysates were simultaneously effected by placing the hydrolysates in flat-bottomed dish in a thin layer in an evacuated desiccator containing solid sodium hydroxide and concentrated sulphuric acid in different dishes. The operation was usually repeated three times to remove most of the acid. The syrupy residue was reconstituted to the original volume with water and aliquots used for

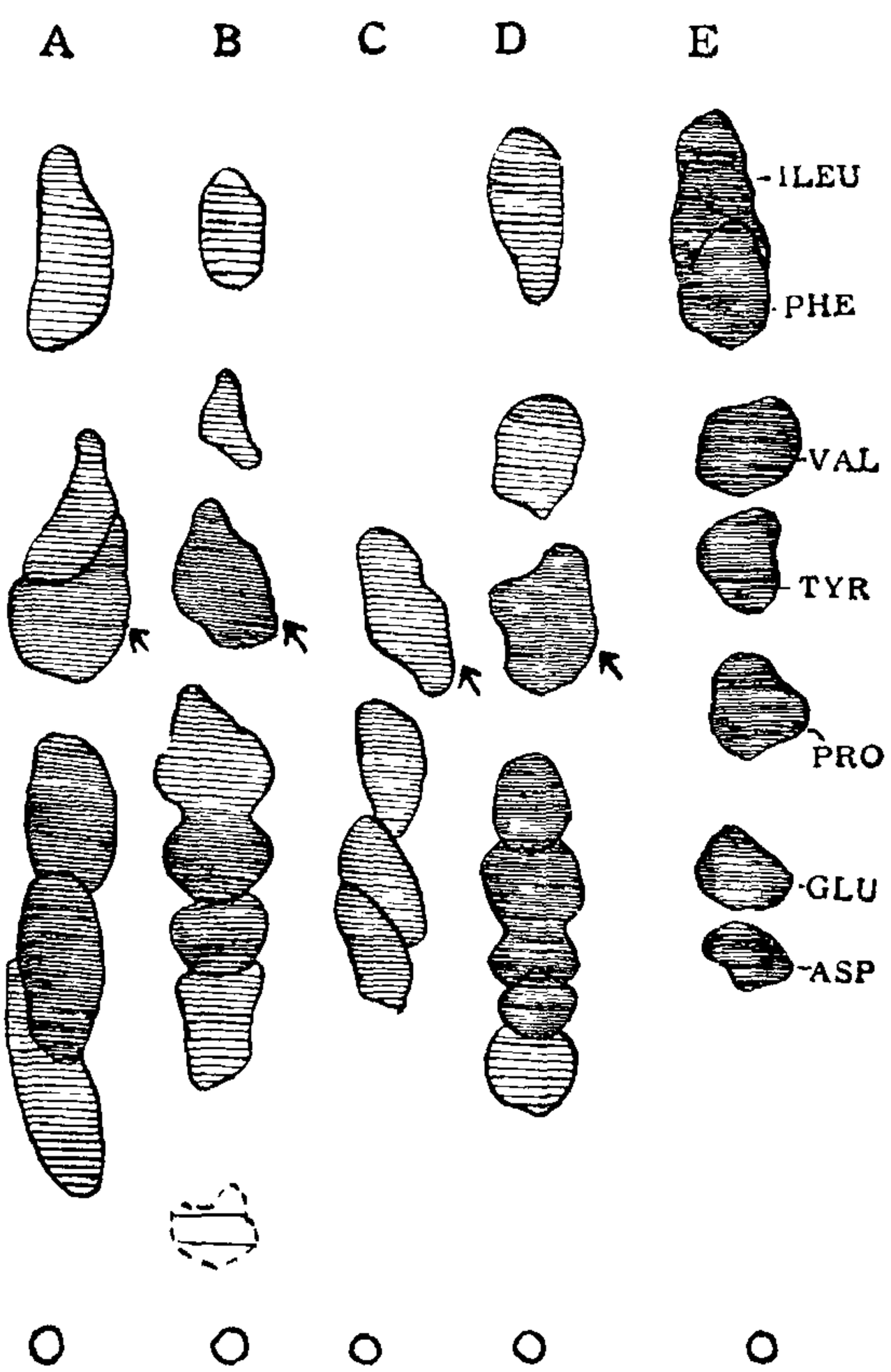

FIG. 2

Chromatograms of hydrolysed extracts of A: Ungrafted variety; B: Raspuri; C: Alfonso; D: Malgova; E: Reference mixture of pure amino acids. Note: Arrow indicates position of the amino acid referred to in text as 'near-tyrosine spot.' Relative intensities of colour spots indicated by shading. Names of amino acids abbreviated according to Brand and Edsall (*loc-cit.*).

chromatographing, on an equal nitrogen basis. The results are represented in Fig. 2.

At 50 × 10^{-3} ml. level of the original extract the chromagram reveals that Mulgova extract shows the maximum number and concentration of amino acids followed closely by the ungrafted country variety and the grafted Raspuri variety. The Alfonso variety shows, surprisingly, the least number and concentration of amino acids.

A careful scanning of the chromatograms leads to the following conclusions:—(1) *Mulgova*: Aspartic acid, glutamic acid, alanine, serine or glycine are the principal amino acids with basic amino acids, valine or methionine and leucines occurring in smaller amounts. Also prominent was another spot (*indicated by an arrow in* Fig 2) near that of tyrosine having a characteristic bright bluish purple colour and hence different from tyrosine which gives the dull purple colour. This is the position reported for γ-amino-butyric acid in a single dimensional chromatogram with butanol-acetic acid solvent.[6] γ-Amino-butyric acid has been reported to be a constituent of many plant tissues.[3,4] Its absolute identity, however, has yet to be established in mango extracts.

(2) *Country variety*: Aspartic and glutamic acids and the near-tyrosine spot are the principal components with glycine, alanine, valine or methionine and leucines as minor constituents.

(3) *Raspuri*: Aspartic and glutamic acids, alanine and the near-tyrosine spot are the principal amino acids and glycine or the basic amino acids, valine or methionine are in smaller amounts. The presence of cystine and leucine group is indicated.

(4) *Alfonso*: Aspartic and glutamic acids and near-tyrosine spot are the chief constituents though the amounts are smaller than that of other varieties. Basic amino acids and alanine are seen in smaller amounts. The picture given by the unhydrolysed extract of this variety, showed the pear-shaped spot which was weak and dull.

The identification of the near-tyrosine spot and an analysis at higher levels by double chromatogram is in progress.

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1. Consdon, R., Gordon, A. H. and Martin A. J. P, *Biochem. J.*, 1944, **38**, 224. 2. Dent, C. E., *Ibid.*, 1948, **43**, 169. 3. —, Stepka, W., and Steward, F. C., *Nature*, 1947, **160**, 682. 4. Joslyn, M. A. and Stepka, W., *Food Research*, 1949, **14**, 459. 5. Govindarajan, V. S. and Sreenivasaya, M., *Curr. Sci.*, 1950, **19**, 39. 6. Reed, L. J., *J. Biol. Chem.*, 1950, **183**, 451.