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A STUDY OF THE FREE AMINO ACIDS IN THE LIQUID ENDOSPERM OF COCONUT (COCOS NUCIFERA)

by

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SUMMARY

On the basis of results obtained with Paper Chromatographic and Thin Layer Ionophoretic techniques, the paper describes the free amino acids in the liquid endosperm of coconut in two stages of maturity.

INTRODUCTION :

The per capita consumption of coconuts in Ceylon is 22.4 kilograms per annum*. At present, the liquid endosperm of the mature fruit may be regarded as an agricultural waste product. Investigations on the nutritional aspects of coconut water have been initiated and this paper describes a study of the free amino acids in the liquid endosperm of coconut.

Studies on the essential amino acids of the coconut endosperm have been reported earlier (Williams 1955, Lyman, 1955, Baptist, 1956). Williams (1955), and Lyman (1955) reported the occurrence of arginine as the major amino acid. They also reported the occurrence of varying amounts of leucine, isoleucine, valine, phenyl alanine, threonine, lysine, histidine, methionine, tryptophan and tyrosine. Baptist (1956) using paper chromatographic techniques showed the occurrence of α alanine and β—aminobutyric acid as the major amino acids in the endosperm of coconut. The occurrence of aspartic and glutamic acids, serine, valine, leucine, isoleucine, threonine and glycine was also shown. Concentrated extracts contained asparagine, glutamine, cystine, the basic amino acids and α—alanine.

EXPERIMENTAL :

Paper chromatographic and thin layer ionophoretic techniques were used.

Two dimensional chromatograms were prepared on Whatman No. 1 paper using the liquid endosperm of mature coconuts (0.25 ml). Concentrated extracts were used for low voltage thin layer ionophoresis (0.05 ml). The extracts were concentrated *in vacuo* usnig a rotary evaporator at a temperature of 60—70° for 3 hours, when an oily liquid was obtained. To the viscous mass formed, an excess volume of purified acetone was added and the mixture allowed to stand for 1 hour in a separating funnel. When two layers could be clearly distinguished, the acetone layer was carefully separated. The remaining fatty mass was dissolved in a minimum volume of distilled water and used for ionophoresis.

* Statistical Abstracts of Ceylon, 1967-68, p. 136.

Chromatography :

One dimensional chromatograms were prepared by the descending technique on Whatman No. 1 paper. The solvents used were as follows : n-Butanol—acetic acid—water (12 : 3: 5 by vol) phenol—water (4: 1 w/v), in the presence of aqueous ammonia and n-butanol-pyridine-water (1:1:1: by vol.). The two dimensional chromatograms were developed in the solvent n-Butanol-acetic acid-water (12:3:5) by vol in the first direction and in phenol-water (4 : 1 w/v) in the presence of aqueous ammonia, in the second direction.

Ionophoresis :

Horizontal low voltage thin layer ionophoresis was conducted on clean glass plates (20 x 10 cm^2) coated with silica gel (0.5 mm thick) in sodium acetate—acetic acid buffer solution (pH 5.5). For the separation of the amino acids a potential of 240 volts was applied for 100 min. while for the separation of glutamine the time of application of 240 volts was reduced to 15 min.

Location reagents :

The ninhydrin reagent was prepared by mixing 0.2 gms. of ninhydrin, 5 ml. of distilled water, 5 ml. of glacial acetic acid and 90 ml. of purified acetone. The papers were sprayed with the reagent, dried and heated for 2—3 min. in an oven at 90°. The amino acids and amines, gave violet colours.

The Erhlich reagent was prepared as follows : 0.2 gms. of p-dimethylamino benzaldehyde, 1 ml of conc-hydrochloric acid and 20 ml. of acetone.

The sulphanilic acid reagent was prepared as follows:—Solution A consisted of 9 gms. sulphanilic acid, 90 ml. of conc. hydrochloric acid and 900 ml. of distilled water. Solution B consisted of a 5% aqueous solution of sodium nitrite. Solution C consisted of a 10% aqueous solution of anhydrous sodium carbonate. Equal volumes of solution A and B were mixed. After a few minutes, to the resulting mixture an equal volume of solution C was added. Histidine and other imidazoles gave characteristic red colours.

Sakaguchi reagent :

The chromatograms were dipped in a 0.1% solution of 8-hydroxy quinoline in acetone, and when the papers were visibly dry, these were dipped in a 2% solution of sodium hydroxide which contained 0.3% of concentrated liquid bromine. Arginine gave an orange-red colour.

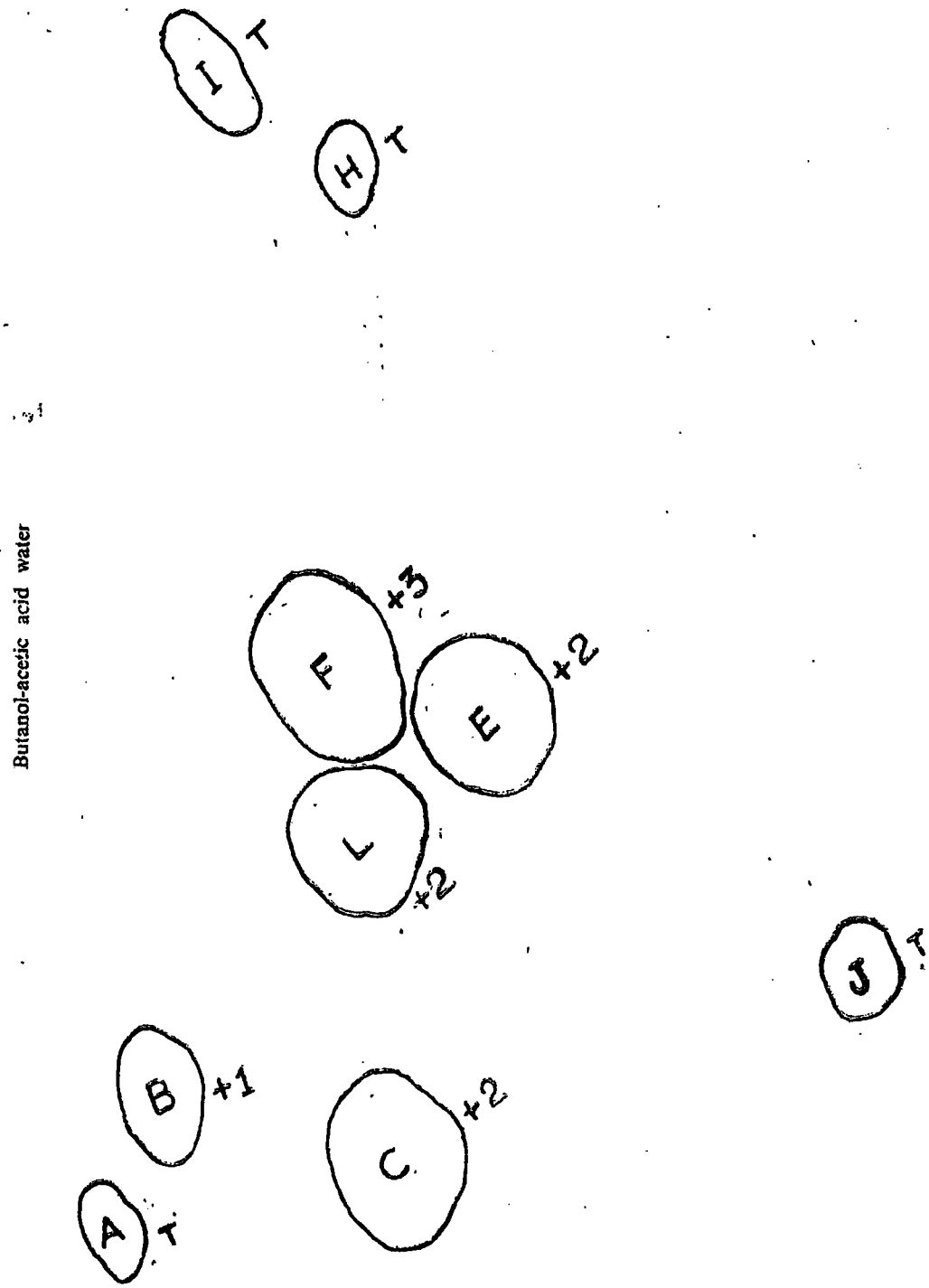
RESULTS AND DISCUSSION :

The relative concentrations of the free amino acids in the liquid endosperm extracts may vary, depending on such factors as the method of extraction, the degree of maturity of the fruit, the season of the year, the soil conditions for growth and also on the stability of the compounds after extraction. Further, the amino acid pattern on a chromatogram is also dependent on the stability of the compounds in the presence of solvents used for development.

A diagrammatic representation of a two dimensional paper chromatogram showing the separation of the free amino acids in the liquid endosperm of mature coconut is illustrated in chart I. <—alanine and glutamic acid occurred in high concentrations. Detectable amounts of aspartic acid, serine, glycine glutamine, valine, <aminobutyric acid, leucine, isoleucine, arginine, histidine and lysine were also seen.

Chart I

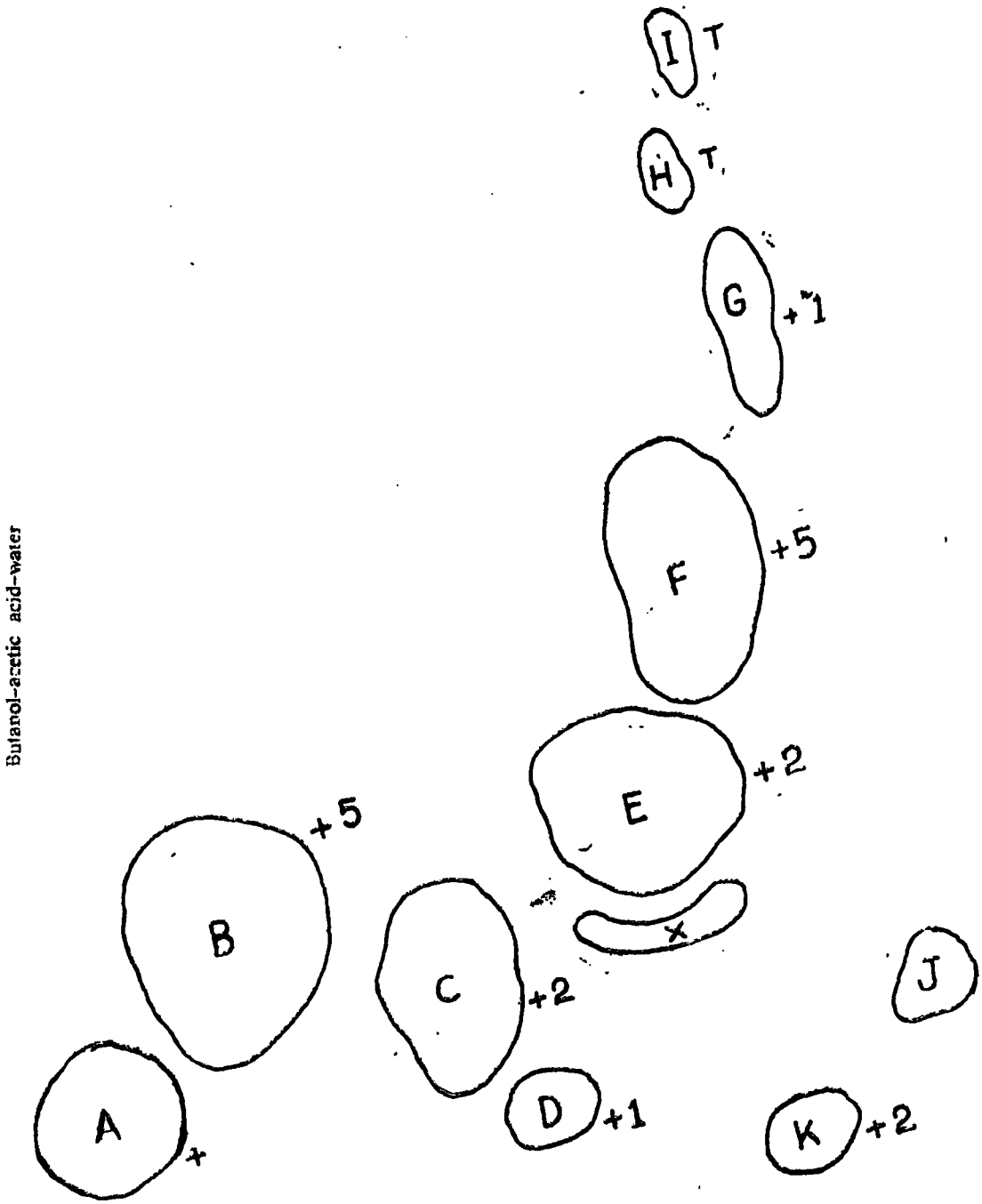
COCONUT WATER (8-10 MTHS)



A diagrammatic representation of a two dimensional chromatogram showing the distribution of the free amino acids in the liquid endosperm of mature coconut. Key to the amino acids is as follows: A-aspartic, B-glutamic, C-serine, D-glycine, E-glutamine, F-alanine, G-Aminobutryic, H-valine, I-leucine and isoleucine, J-arginine, K-histidine and lysine and X-unknown. The numerals indicate the relative concentrations by visual observations. An intensity of 1 is approximately equivalent to 0.4 x 10^{-5}g.

Chart II

YOUNG COCONUT WATER



A diagrammatic representation of a two dimensional chromatogram showing the distribution of the free amino acids in the liquid endorperm of young coconut. Key to the amino acids is as follows: A-aspartic, B-glutamic, C-serine, E-glutamine, F-alanine, H-valine, I-leucine, and isoleucine, J-arginine, L-threonine. The numerals indicate the relative concentrations by visual observations. An intensity of 1 is approximately equivalent to 0.4 x10-5g.

In the liquid endosperm of the immature coconut (5-6 months), the concentration of the total amino acids was less than that in the mature (7-9 mths.) coconut. A diagrammatic representation of the free amino acids has been illustrated in Chart II. An appreciable amount of threonine was detected. In comparison with the amino acids in the liquid endosperm of immature king coconut (5-6 mths.), an interesting feature was the presence in appreciable amounts of the neutral amino acids serine, threonine and glycine in the king coconut. Traces of phenylalanine was also detected. In the liquid endosperm of immature coconut the occurrence of histidine was confirmed by co-chromatography and by the characteristic colour given with the diazotised sulphanilic acid reagent. It was also observed that another compound occurred which had a lower Rf value in the Butanol-acetic acid-water solvent (12 : 3 : 5) and which gave an identical colour reaction as histidine. Low voltage thin layer ionophoresis followed by the use of the colour reagent, diazotised sulphanilic acid clearly showed the occurrence of histidine. The use of the Sakaguchi reagent on the paper chromatograms and on the thin layer ionophoresis plates clearly showed the presence of arginine but not in very high concentrations. The occurrence of trytophan was confirmed by the use of Erhlich reagent and that of lysine by thin layer ionophoresis. Among the amines, the occurrence of glutamine in appreciable amounts was a notable feature and it was confirmed by thin layer ionophoresis.

In a few chromatograms, the occurrence of piperiolic acid was detected by the characteristic reddish fluorescence given, when the papers treated with ninhydrin were examined under U.V. (3650). This observation could not be repeated with subsequent extracts probably due to the rapid changes in the amino acid concentration during maturity of the fruit.

A comparison between the free amino acids in the liquid endosperms of immature coconut showed a similar pattern, with a slight variation in the concentration of some neutral amino acids. The concentrations of serine, threonine and glycine were higher in the liquid endosperm of king coconut.

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