

Protein and Free Amino Acids of *Citrullus colocynthis*

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The protein contents in the seeds of ripe and unripe fruits and the free amino-acids in the pulp, seeds and protein hydrolysate of *Citrullus colocynthis* have been described.

CITRULLUS *colocynthis*, is locally known as 'Tumba', belongs to N.O. Cucurbitaceae. It is a large trailing herb with yellow flowers common on loose sandy soil. The fruit and seeds are used as purgative while the root is used as antidote to snake poison, in jaundice and urinary diseases¹. The seeds are rich in oil contents and are also reported to contain phytosterol, alkaloids and glycosides.²

Citrullus colocynthis grows wildly and abundantly in Rajasthan desert and is used commonly in the local areas as fodder. An attempt is, therefore, made to investigate the protein and free amino-acid contents of its seeds and pulp.

The protein percentage of the ripe seeds, calculated from the modified Kjeldahl's method of nitrogen estimation³, was found to be 25.6% and that in the pulp and unripe seeds to be 17% and 15% respectively. The distillation of ammonia from digested material was carried out in a one piece steam distillation apparatus⁴.

The protein percentage was also determined spectrophotometrically using Biuret Method⁵. The results obtained by this method agreed with the earlier one.

For the study of free amino-acid contents, the defatted seeds were extracted with 10% Sodium Chloride (A.R.) solution, brought to pH 7 by a phosphate buffer at a meal, and the protein was removed completely by precipitating with 10% tri-chloro-acetic acid. The centrifugate was chromatographed on Whatman filter paper no. 1 in the phenol:water (3:1) system and developed by ninhydrin spray.

The pulp, ripe and unripe seeds show characteristic pattern and concentration of amino-acids.

1. *Ripe seeds*: The amino-acid present in it was identified by finger printing technique and shown to be valine only. The R_f value agreed with that of valine.

2. *Un-ripe seeds*: The study of the chromatogram of unripe seeds showed the presence of five free amino-acids. The amino-acids were identified by finger printing technique and were found to be

glutamic acid, histidine and valine. The remaining two spots could not be identified by using various available reference amino-acids.

3. *Pulp*: The chromatogramme of pulp showed the presence of Arginine as identified by the same technique.

4. *Protein hydrolysate*: The protein residue as obtained above i.e., by precipitation with 10% trichloro acetic acid was hydrolysed with 5.5N HCl in sealed glass tubes till negative to biuret test. The acid-free hydrolysate after repeated distillation in vacuo was subjected to paper chromatographic analysis. The chromatography of free amino-acids revealed the presence of the same five amino-acids as were obtained in case of unripe seeds.

It is interesting to note that the pulp showed the presence of only one amino-acid (arginine) which was not identified in the unripe as well as ripe seeds of the plant. While the ripe seeds contained only valine; the unripe seeds showed the presence of four more amino-acids besides valine. The five amino-acids present in the unripe seeds were also identified in the protein hydrolysate of the ripe seeds.

It is therefore apparent that amino-acids of unripe seeds were used in protein formation and except valine, rest four of the amino-acids have been completely consumed in the process.

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