STUDIES ON THE LEAF JUICE OF TRIFOLIUM RESUPINATUM

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The changes in the protein and lipid contents of the juice extracted from *Trifolium resupinatum* (Persian clover) leaves, when incubated at 37°C for different periods, were studied. A 4.6% increase in TCA (trichloroacetic acid) soluble nitrogen and 5.8% in free phosphates associated with a 3.45 and 2.08% decrease in phospholipids and nucleic acid phosphorus respectively, was observed after 2 hr.

The juice, when incubated at 37°C, in a medium containing 0.8% (w/v) casein at pH 5.5, hydrolysed 49.0% of the casein. The juice also liberated inorganic phosphorus from egg lecithin and hydrolysed 38.78% of it in 24 hr under optimum conditions.

Introduction

The juice extracted from green leaves contains a fair amount of proteins and lipids, as well as hydrolytic and proteolytic enzymes. These enzymes affect the extractability and shelf-life of the proteins. The presence of proteolytic enzymes in the green leaves of non-latex bearing plants was reported by Loew.[1] A decrease in the amount of extractable proteins, from plant juices, was studied by various workers.[2–4] Hydrolysis of phospholipids by the aqueous extract of fresh carrot or cabbage leaves was reported by Hanahan and Chaikoff[5,6] and confirmed by Rose,[7] Acker *et al.*[8] and Ducet.[9] Liberation of inorganic phosphates from phosphilipids by carrot juice and tobacco leaves chloroplasts was observed by Holden.[10] Kates[11,12] reported the liberation of inorganic and water-soluble organic phosphates from egg lecithin by aqueous extracts of plant tissues. The lecithinase activity was also observed in latex serum of *Herea brasilienses.*[13]

The work on the extractability of proteins from various leaves[14] showed that *Trifolium resupinatum* was the best crop for the extraction of proteins. However, it was observed that the amount of non-proteinous nitrogen and free phosphates increased substantially during processing. The changes in the juice of *Trifolium resupinatum* leaves, stored for various periods, is reported in this paper.

Materials and Methods

The leaves of *Trifolium resupinatum* were collected from the local field; tough stems were removed and about 200 g of leaves were minced in a domestic meat mincer. The sap was squeezed out by hand through double thickness of cheese cloth. The juice thus extracted was employed in the experiments.

Autolytic Breakdown of Protein.—One volume of the juice was diluted with four volumes of distilled water. Five samples, each containing 2 ml of the diluted juice were incubated at 37°C for 30, 60, 90 and 120 min in the presence of chloroform as preservative. The proteins were precipitated with equal volume of 10% TCA. The supernatant of each sample was collected and its nitrogen content was determined.

Changes in the Phosphorus Content of the Juice.—Five samples, each containing 5 ml of diluted juice, were incubated at 37°C. The samples were taken after every 30 min and the phosphorus compounds were fractionated by the method of Holden,[10] with the modifications suggested by Singh,[15] and the phosphorus content was estimated.

Hydrolysis of Lecithin.—Hydrolysis of lecithin by the leaf juice was studied by the method suggested by Kates.[11] Five samples each containing 1 ml of the undiluted juice and 4 ml of 0.625% lecithin solution (w/v) at pH 5.5 were incubated at 25°C. The samples were collected after 30 min intervals, centrifuged and the supernatants were examined for total and inorganic phosphorus. The blanks were also run simultaneously.

Chemical Estimation.—Nitrogen was estimated by the microkjeldahl method. Total and inorganic phosphorus in all the samples was estimated by the method of Holden and Pirie.[16] The nucleic acid phosphorus was measured spectrophotometrically by the method of Spirin.[17]

Results and Discussion

Extraction of protein from juice involves various steps which take about 90–120 min and the temperature rises up to 37°C during processing.[4] During summer, room temperature also rises and at times it is 37–40°C. Figure 1 shows the percentage breakdown of proteins at 37°C at different periods. A decrease of 4.5% in the proteinous nitrogen was observed after 2 hr. Protein autolysis in the *Trifolium resupinatum* leaf juice was found comparatively much lesser than all the leaf extracts studied by Singh,[4] who has reported 7–40% loss in the proteinous nitrogen in various extracts. In this respect the exploitation of *Trifolium resupinatum* leaves for bulk production of protein seems more profitable.

The proteolytic enzymes present in the juice liberated 9.87% of non-protein nitrogen from casein under optimum conditions after 2 hr (Fig. 2).

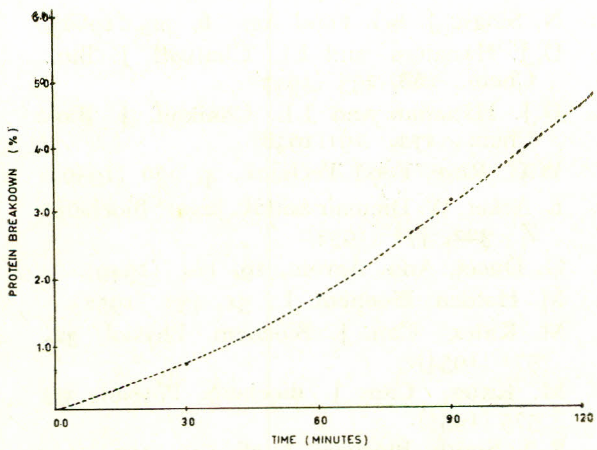

Fig. 1.—Protein autolysis of *Trifolium resupinatum* juice.

Figures 3 and 4 present the time course curves for the percentage decrease in the lipid and the nucleic acid phosphorus, and the percentage increase in the soluble phosphorus when the extract was incubated at 37°C without any substrates. The enzymes present in the extract liberated 2.0% lipid phosphorus and 3.37% nucleic acid phosphorus thus increasing the amount of soluble phosphorus in aqueous phase. This increase in soluble phosphorus was accompanied by a similar increase in soluble nitrogen (Fig. 1).

Figure 5 shows the liberation of inorganic and the soluble organic phosphorus from egg lecithin by the juice. The curve showing liberation of inorganic phosphorus is linear over the period studied. The juice liberated 40% of the phosphorus from lecithin as inorganic phosphorus

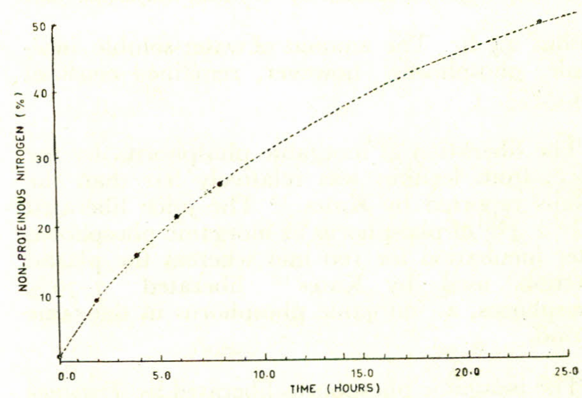

Fig. 2.—Casein hydrolysis by *Trifolium resupinatum* juice.

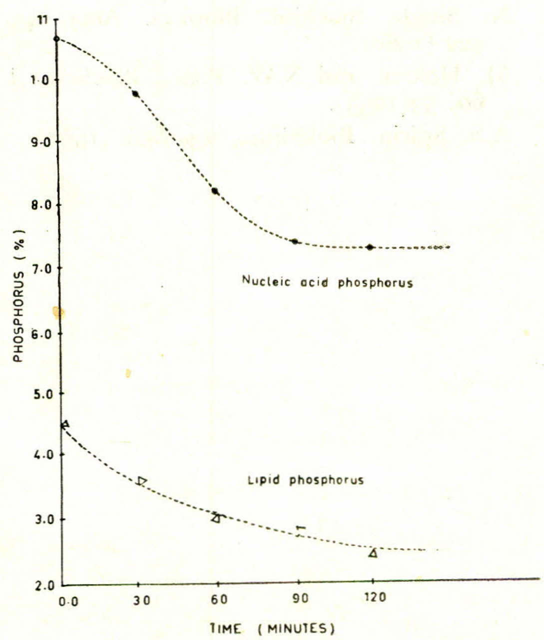

Fig. 3.—Decrease in nucleic acid and lipid phosphorus due to autolysis of *Trifolium resupinatum* juice

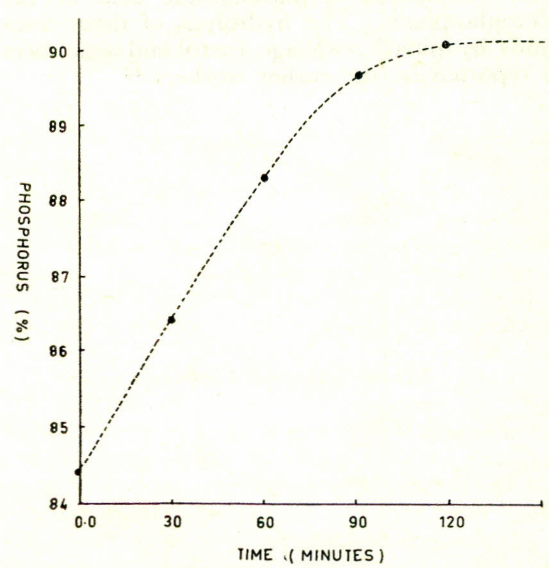

Fig. 4.—Increase in soluble phosphorus during autolysis of *Trifolium resupinatum* juice.

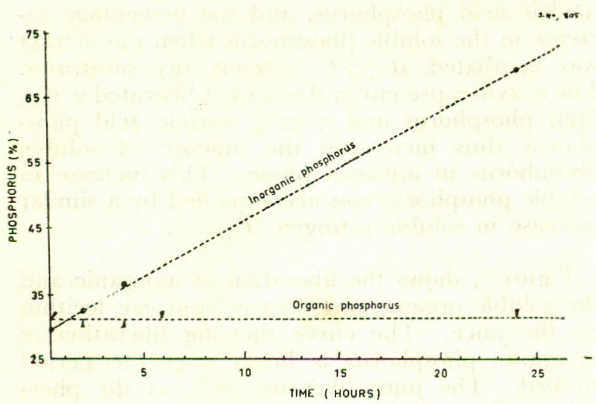

Fig. 5.—Hydrolysis of lecithin by *Trifolium resupinatum* juice.

within 24 hr. The amount of water-soluble inorganic phosphorus, however, remained constant (31.5%).

The liberation of inorganic phosphorus, by the juice, from lecithin was relatively less than the results reported by Kates.[12] The juice liberated only 2.4% of phosphorus as inorganic phosphorus after incubation for 100 min whereas the plastid fraction used by Kates[12] liberated 7–70% phosphorus, as inorganic phosphorus in the same period.

The inorganic phosphorus liberated by *Trifolium resupinatum* juice from lecithin seems either due to the breakdown of phosphotidic acid or the glycerophosphate. The hydrolysis of these compounds by spinach, cabbage, carrot and sugar beet was reported by the earlier workers.[12]

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