DIGESTIBILITY OF THE LEAF PROTEIN CONCENTRATES

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The enzymic digestion of leaf protein concentrates was carried out with trypsin, pepsin, *torula* yeast; and the enzymes present in the aqueous extracts of the berries of *Withania coagulans* and ox pancreas. The digestibility of the samples was determined after 3, 6, 9 and 24 hours.

Leaf proteins showed high rate of digestion with trypsin, pepsin and the enzymes present in the aqueous extract of ox pancreas. The enzymes present in *torula* yeast and the berries of *Withania coagulans* showed poor proteolytic activity.

Introduction

Grass, is the largest single crop in the world. The changes in the flavour and texture that the animals bring about in the proteins present in the leaves of grass is very much liked by most of the people. This advantage is, however, gained at the expense of heavy losses because the overall efficiency of animals when turning plant material into human food is usually 5-10% and is never greater than 30%.[1]

Although the process for the extraction of proteins from leaves has been thoroughly studied,[2] the digestibility and nutritive values of these proteins have received little attention. The amino-acid composition of leaf protein concentrates (LPC) has been reported to be as good as or better than that of many foodstuffs.[3] This indicates that plant leaves may be an important source of food for human consumption. However, amino acid composition alone may not give a true picture as digestibility may effect the nutritive value of LPC. The chemical decomposition during acidic or alkaline digestion and the loss of certain essential amino acids make these methods unreliable.[4] Enzymic methods of digestion have least effect on the composition of the final products. The action of proteolytic enzymes on pure proteins [5,6] and on proteins in combination with complex materials as they occur in nature have been studied by various workers[7–9] but little has been accomplished on the action of proteolytic enzymes on leaf proteins. However, a notable contribution has been recently made by Akeson and Stahmann.[10] This work was undertaken to study and compare the action of various proteolytic enzymes of plant and animal origin on LPC.

Experimental

Extraction of Leaf Proteins.—Young leaves of Shatalla (*Trifolium resupinatum*), Rawan (*Lathyrus aphaca*) and from a mixed crop of sarson (*Brassica campestris*), Shatalla, methi (*Trigonella foenum graceum*) and Jai (*Avena sativa*) were stripped from tough stems. The proteins were extracted by the procedure used by Crook and Holden.[11] Leaves were separately minced in a domestic electric mincing machine and the pulp squeezed through a fine cloth. A second extract was made by adding a suitable amount of water to the residue; remincing and squeezing again. Proteins were precipitated by heating the juice to 80°C. The coagulum was transferred to long cloth bags and excess of water was removed by hand-pressing. The protein cake thus obtained was repeatedly washed with water and hand-pressed till the liquid issuing out was clear. The protein samples thus obtained were placed in polythene bags and stored in a freezer at-10°C.

Determination of Dry Matter and Nitrogen Content.—Dry matter (DM) was determined by heating the homogenised protein sample at 100°C for 40 hr.[12] Nitrogen content determinations were made by micro-Kjeldahl procedure using copper–selenium catalyst. Proteinous nitrogen (PN) and non-proteinous nitrogen (NPN) in the samples was estimated as trichloroacetic acid (TCA)—insoluble and TCA-soluble N respectively. The protein contents of the samples were calculated as protein N × 6.0.[13]

Determination of Digestibility.—Trypsin (B.D.H.), pepsin (Merck), torula yeast (*Candida utilis*), the enzymes present in the aqueous extracts of the berries of *Withania coagulans* and ox pancreas were used for the determination of the digestibility of leaf-proteins.

One g of the homogenised LPC sample was suspended in 20 ml of the buffer solution in 50-ml flasks. The samples, after addition of suitable concentrations of the enzymes, were incubated at an optimum temperature and were removed after 3,6,9 and 24 hr. The proteins present in 2 ml of the suspension were precipitated by the addition of 2 ml 20% TCA and were separated by centrifuging at 2500 x g for 15 min. The N-content of the supernatent (NPN) was determined. The

NPN x 6.0 is referred to in the text as digestible protein. Blanks as well as the autolysis of the enzyme during the experiment were also determined.

Trypsin.—1 g of LPC (DM = 32.23%) was suspended in 20 ml of the citrate buffer (pH 7.0) and incubated at 37°C after the addition of 1,2 and 5 ml 2.5% trypsin. [14,15]

Pepsin.—1 g of LPC (DM=27.94%) was suspended in 20 ml of the acetate buffer (pH = 1.8) and incubated at 37°C [20] after the addition of 2 and 5 ml of 3%, or 5 and 10 ml of 6% pepsin solution.

Torula yeast.—1 g of LPC (DM=28.16%) was suspended in 20 ml of the citrate buffer (pH =4.5) and incubated at 30°C [16,17] after the addition of 1,2 and 5 ml 3% torula yeast.

Withania coagulans.—100 g of the berries were ground to 40 mesh and made into paste with about 80 ml distilled water. This paste was centrifuged and the top brown liquid removed. The residue was washed twice with water. 1 g of the LPC (DM = 33.15%) was suspended in 20 ml of the citrate buffer and incubated at 50°C[18,19] after the addition of 1 ml extract (1 g of *Withania coagulum/ml*). Optimum pH was determined to be 3.0.

Ox pancreas.—600 g of frozen pancreas were cut, minced and blended with ice-cold water in an electric blender and quickly filtered. The process was repeated twice and 800 ml of the extract was obtained. One gram of LPC (DM = 26.95%) was suspended in 20 ml of the citrate buffer (pH = 7.0) and incubated at 37°C[20] after the addition of 1 and 2 ml of extract.

Discussion

The proteolytic activity of each enzyme was determined by the rate of conversion of proteinous nitrogen (PN) of the leaf protein concentrate into non-proteinous nitrogen (NPN).

The optimal activity of trypsin can be measured over a wide range of pH.[15] As the proteolytic activity of trypsin was to be studied for long periods, the experiments were carried out at optimum pH of 7.0. 24.0% of the leaf-protein extracted from the mixed crop was digested by trypsin after 9 hr of incubation (Table 1). However, the amount of NPN decreased when the process was carried out for 24 hr. This decrease in the NPN can be attributed to the fact that the leaf protein contains 20–25% of highly unsaturated lipids which get oxidised in the presence of chlorophyll. The oxidised lipids get polymerised in the presence of proteins. It has also been reported that either the amino acids copolymerise with oxy-polymerising lipids or become occluded in them.[21,22] As this process is a function of pH and is facilitated at higher pH (7.0) the NPN decreased in case of trypsin.

TABLE 1.—PERCENTAGE DIGESTIBILITY OF LPC FROM MIXED CROP.

Time hr	Trypsin			Pepsin			
	25 mg	50 mg	125 mg	60 mg	150 mg	300 mg	600 mg
3	6.08	8.06	10.96	2.69	3.25	7.63	9.20
6	8.71	10.91	15.80	4.65	4.92	9.95	15.00
9	13.09	14.70	23.96	5.56	7.92	13.82	20.06
24	11.39	14.02	20.40	9.37	14.20	19.77	25.57

TABLE 2.—COMPARATIVE PERCENTAGE DIGESTIBILITY OF LPC.

Time in hr	Trypsin 125 mg			Pepsin 600 mg			Pancreatic extract		
	Mixed crop	Shatala*	Rawan**	Mixed crop	Shatala*	Rawan**	Mixed crop	Shatala*	Rawan**
3	10.96	6.04	13.76	9.20	1.03	3.80	5.17	19.12	20.88
6	15.80	14.37	22.03	15.00	5.18	8.00	12.03	22.74	33.48
9	23.96	16.94	31.47	20.06	6.39	12.19	17.94	26.89	46.07
24	20.40	17.60	39.77	25.57	7.51	25.48	38.75	50.71	64.94
36	—	—	66.87	—	—	40.86	—	—	84.88
48	—	—	56.16	—	—	52.10	—	—	76.49

* Trifolium resupinatum. ** Lathyrus aphaca.

TABLE 3. PERCENTAGE DIGESTIBILITY OF LPC FROM MIXED CROP.

Time hr	Torula yeast			Withania coagulans extract	Pancreatic extract	
	30 mg	60 mg	150 mg	1 ml	1 ml	2 ml
3	1.00	1.73	4.89	3.92	2.76	5.17
6	1.77	3.17	5.74	6.46	8.21	12.03
9	2.78	3.89	6.90	7.06	11.85	17.94
24	3.15	4.09	7.73	10.36	21.76	38.75

The digestibility of the proteins extracted from Rawan and Shatalla leaves was 39.8 and 17.6% after 24 hr. The decrease in the NPN in case of these proteins was observed when the samples were incubated beyond 36 hr (Table 2).

Pepsin did not show any exceptional behaviour as observed in case of trypsin. The rate of digestion in case of the proteins extracted from the mixed crop was found to be comparatively high. 25.6% of the leaf protein was digested after 24 hr incubation. However, the proteins extracted from Rawan and Shatalla leaves showed 25.5 and 7.5% digestibility after 24 hr (Table 2).

Torula yeast released only 7.7% of the total PN after 24 hr of incubation (Table 3). Although yeast possesses a store of proteolytic enzymes[23] this seems to be an exceptionally low rate of digestion. The weak proteolytic activity seems to be due to the adsorption of proteolytic enzymes by yeast cells,[24] thus making the enzymes unavailable for the proteolysis. It is believed that these enzymes penetrate through the cell membrane and are fixed on the element of protoplasm.

Pancreatic extract showed the maximum rate of digestion for all the varieties of LPC. 38.8, 64.9 and 50.7% of the PN present in mixed crop, Rawan and Shatalla was released after 24 hr of incubation (Table 2). As the proteolysis in case of pancreatic extract was carried out at pH 7.0, a decrease in NPN has also been observed as in case of trypsin.[25] The pancreatic extract seems to be more suitable for the digestion of LPC. The extract is cheap and can be obtained in large quantities from pancreas. Moreover, powdered trypsin and pepsin have been found to be pyrogenic while the use of pancreatic extract results in a decrease of pyrogens[4] and a more rapid hydrolysis of LPC. The enzymes present in *Withania coagulans* berries on the other hand showed weak proteolytic activity (Table 3).

On the basis of these results it can be safely assumed that the LPC prepared from leaves would be easily digested by human system.

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