THE RÔLE OF PEPSIN IN THE *in vitro* DIGESTION OF PROTEINS.

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For the quantitative study of the digestibility of proteins, the merits of the *in vitro* method of Waterman and Johns (*J. Biol. Chem.*, 1921, **46**, 9; 1921, **47**, 285; 1923, **56**, 501) are being increasingly recognised. The method affords a convenient means of comparing the digestibility of proteins without resorting to the time-consuming experiments on laboratory animals. It involves the measurement of the increase in amino nitrogen accompanying the treatment of the protein, first with pepsin and subsequently with trypsin. Sreenivasaya and Sreerangachar (*Biochem. J.*, 1935, **29**, 291) employing the dilatometer, showed that the digestibility of the proteins could be ascertained by measuring the volume change resulting from the hydrolysis of the protein by trypsin alone. (See also Bhagvat and Sreenivasaya, *Proc. Ind. Acad. Sci.*, 1935, **2B**, 316). Wewers (*Angewandte Chemie*, 1934, **47**, 822) in the study of the digestibility of blood and fish meals, employed pepsin. Bhagvat and Sreenivasaya (*Journ. Ind. Inst. Sci.*, 1936, **19A**, 9) showed that higher digestibility values were obtained for the total globulins of cowpea (*Vigna catiang* Walp.) and aconite bean (*Phaseolus aconitifolius* Jacq.) when the proteins were subjected to the successive action of pepsin and trypsin than when they were treated with trypsin alone. It would appear that for evaluating the true digestibility of proteins by the *in vitro* method, it is important to maintain the strict *in vitro* sequence of enzyme action as was originally suggested by Waterman and Johns, but the exact rôle of pepsin in these experiments still remains obscure.

In the course of our work on the digestibility of pulse proteins in their natural state, it was observed that pepsin treatment rendered a strikingly large part of the nitrogen of the pulse flour, soluble. It was of interest to ascertain the extent of solubilisation accompanying the treatment with pepsin of various food grains, and the present paper deals with the results obtained in such an enquiry.

EXPERIMENTAL.

The following materials have been studied:—Bengal gram (*Cicer arietinum* Linn.); Green gram (*Phaseolus mungo*); Horse gram (*Dolichos biflorus*); Soya bean (*Glycine hispida*); Black gram (*Phaseolus radiatus*); Field bean (*Dolichos lablab*); Peas (*Pisum*

sativum) and Dhal (*Cajanus indicus*). The food grains were all purchased from the local market, dried in the sun and powdered to pass through a 100 mesh sieve.

5 gm. of the flour were suspended in 50 c.c. of 0.05N HCl and mixed with 5 c.c. of a 1.0 per cent. solution of pepsin (Pfansteihl's) also prepared in 0.05N HCl. After mixing, an aliquot of 10 c.c. was pipetted out and the total nitrogen estimated by the Kjeldhal method. (Duplicates agree within 0.5 per cent.) The mixture was then incubated at 30°C. with frequent shaking for 48 hours, after which period it was centrifuged and the total nitrogen in 10 c.c. of the clear liquid estimated. A parallel experiment, in which 5 c.c. of the boiled enzyme solution was substituted in place of the active solution, served as a control. All estimations were carried out in duplicate and the average values are tabulated below. (Table I.)

TABLE I.

Material	Total N in mixture mgm.	Control Clear centrifugate mgm. N.	Exptl. Clear centrifugate mgm. N.	Increase due to pepsin mgm. N.	Percentage solubilisation of nitrogen
Bengal gram (*Cicer arietinum* Linn.) ..	34·2	13·4	29·8	16·4	87·1
Green gram (*Phaseolus mungo*)..	36·6	15·0	18·3	3·3	49·9
Horse gram (*Dolichos biflorus*) ..	37·0	15·7	24·6	8·9	66·5
Soya bean (*Glycine hispida*) ..	53·1	14·0	20·9	6·9	55·3
Black gram (*Phaseolus radiatus*)	33·4	7·9	16·0	8·1	47·9
Field bean (*Dolichos lablab*) ..	37·9	11·2	27·7	16·5	57·3
Peas (*Pisum sativum*)	34·9	10·8	25·8	15·0	73·9
Dhal (*Cajanus indicus*)	31·4	13·0	16·9	3·9	54·1

The quantity of nitrogen solubilised under the conditions of the experiment is not appreciably affected by varying the proportion of the meal to the enzyme. Table II gives the results obtained with Bengal gram (*Cicer arietinum*).

TABLE II.

Ratio of flour to enzyme	Total N in the mixture in mgm.	Control Clear centrifugate mgm. N.	Exptl. Clear centrifugate mgm. N.	Increase due to pepsin action
100 : 1·0	34·2	13·4	29·8	16·4
100 : 1·5	34·2	13·3	30·0	16·7
100 : 2·0	34·0	13·5	30·3	16·8

DISCUSSION.

The results given in Table I show clearly that pepsin exerts a considerable solubilising action on the nitrogen of the material and clearly brings out the importance of the pepsin treatment in the *in vitro* study of digestibility. Different food materials are influenced to different extents and such differences are probably related to variations in the digestibilities of the grains.

Pepsin treatment affects subsequent hydrolysis by tryptic enzymes by influencing both the nature and the quantity of the substrate provided for their action. Studies on the rate of liberation of the individual amino acids from food materials treated with trypsin + kinase alone, and from the same materials treated with trypsin + kinase after a preliminary digestion with pepsin, should be of value. Experiments using active trypsin in place of pepsin show that this enzyme exerts but little solubilising action.

It may be remarked here that with isolated proteins, the difference between the 'pepsin accompanied by trypsin' and 'trypsin alone' digestions, was not large. But when working with whole meals, striking differences between the two treatments were noticed. This emphasises the imperative need for pepsin digestion in studies of the *in vitro* digestibilities of food materials. It may also be mentioned that pepsin exerts action on the proteins of the food grains even though they are present in the solid phase and not dissolved or peptised by the medium employed.

The solubilisation by pepsin is definitely due to proteolysis and not due to disaggregation as there is a definite increase in the amino nitrogen due to this treatment and is similar to the effect exerted by the yeast enzyme on wheat proteins reported by Blagoveschentski and Yurgenson (*Biochem. J.*, 1935, **29**, 805).

SUMMARY.

Pepsin exerts a definite solubilising action on the proteins of the food materials. Such action is due to proteolysis.

The extent to which the proteins are solubilised vary with different materials. Among the materials studied, Bengal gram (*Cicer arietinum* Linn.) gives the highest value (87.1 per cent.) and Black gram (*Phaseolus radiatus*) the least (47.9 per cent.).

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