Enzymatic Modification of the Extractability of Nitrogenous Constituents from Pea Beans

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Cellulase 36, a food grade enzyme preparation derived from *Aspergillus niger*, was found to be capable of enhancing the extractability of nitrogenous constituents from pea beans. The exact amount of nitrogen extracted was apparently dependent upon several factors, such as sodium acetate buffer concentration, enzyme concentration, substrate concentration, pH, incubation time, and NaCl concentration. The increase in the amount of nitrogen extracted appeared to be a result of the enzymatic hydrolysis of both bean proteins and carbohydrates. The enzyme was also capable of increasing the rate of filtering the aqueous extract of pea beans.

Pea beans contain approximately 25% protein of reasonably good quality, but their utilization for human consumption in the United States is low and declining because of excessive time required for preparation. Several processes have been developed for preparing precooked and quick-cooking pea beans (Dorsey *et al.*, 1961; Feldberg *et al.*, 1956; Rockland and Metzler, 1967; Steinkraus *et al.*, 1964).

The use of microbial enzymes to solubilize seed proteins has been reported. Yasumatsu *et al.* (1966) found that the enzymes of *Trametes sanguinea* are capable of solubilizing yeast cells and soybean meal efficiently. Abdo and King (1967) reported more efficient extraction of proteins from soybeans treated with the enzymes produced by *Pestalotiopsis westerdijkii*. Recently, Sreekantiah *et al.* (1969) showed that the commercial enzyme preparation obtained from *Trametes sanguinea* is very effective in hydrolyzing sesame meal and chickpea. This report demonstrates that Cellulase 36, a food grade enzyme preparation derived from *Aspergillus niger*, is capable of enhancing the extractability of nitrogenous constituents from pea beans. Data on the enzymatic improvement of the rate of filtering the aqueous extract of pea beans are also included in this report.

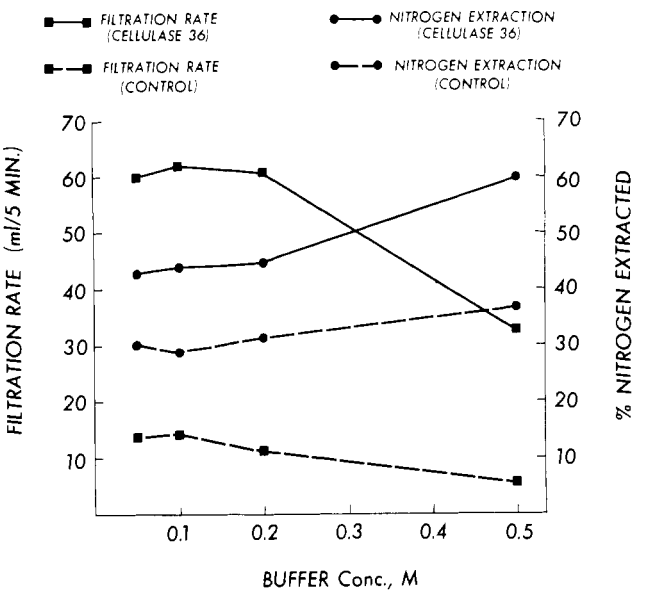

Figure 1. Effect of buffer concentration. Experimental procedure was the same as the standard method except that different buffer concentrations were used

EXPERIMENTAL

Certified pea beans used throughout this work were purchased from Agway, Inc. (Geneva, N.Y.). The seeds were prepared for the enzyme studies by grinding them through the 1B screen of a Fitz mill, Model D (W. J. Fitzpatrick Co., Chicago, Ill.).

Cellulase 36, used in this study, is a commercial food grade enzyme preparation derived from *Aspergillus niger* (Faith, 1969). Its cellulolytic activity on cellulose (Solka Floc) and carboxymethyl cellulose has been reported (Rohm and Haas Co., 1964).

The standard extraction procedure involved the blending of 5 g of ground beans with 100 ml of 0.1*M* sodium acetate buffer (pH 4.0) in a Waring Blendor for 3 min. To this mixture 100 mg of enzyme and 1 ml of toluene were added. The contents were then thoroughly mixed and incubated at 43° C for 22 hr. Controls were prepared exactly in the same manner, except that no enzyme was added. Filtration rate was determined by filtering the reaction mixture through Whatman No. 2v folded filter paper in a 100-mm funnel. Under the same conditions, the filtration rate of distilled water was found to be 94.7 ml per 5 min. Total nitrogen of the filtrates was analyzed by a slight modification of the standard micro-Kjeldahl method (AOAC, 1960), replacing mercuric oxide and potassium sulfate with a Kjeldahl tablet containing sodium sulfate and selenium as the catalyst (The British Drug Houses, Ltd., Poole, England). The results of nitrogen extraction are expressed as % total nitrogen extracted from the beans. The nitrogen contributed by enzyme itself has been substracted from that of appropriate extracts. Nonprotein nitrogen was determined by the method of Becker

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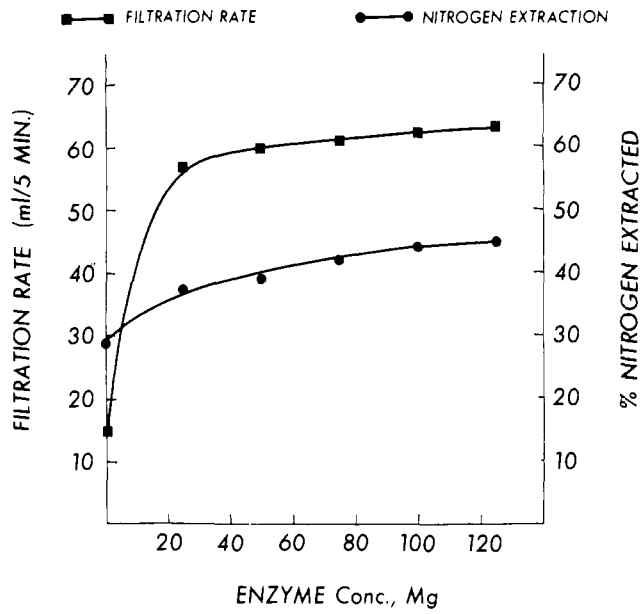

Figure 2. Effect of enzyme concentration. Experimental procedure was the same as the standard method except that different enzyme concentrations were used

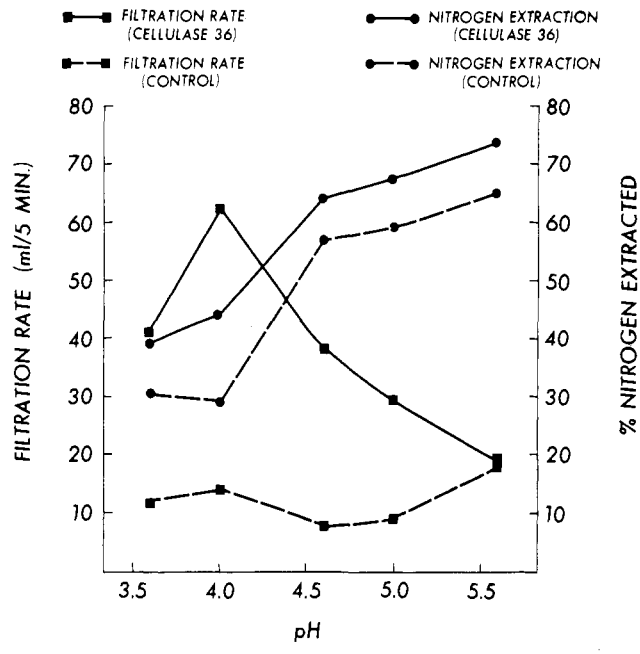

Figure 4. Effect of pH. Experimental procedure was the same as the standard method except that 0.1*M* sodium acetate buffer at different pH was used

et al. (1940). Sugar determination was carried out as described by Shallenberger and Moores (1957).

RESULTS AND DISCUSSION

The effect of buffer concentration on the enzymatic improvement of nitrogen extraction and filtration rate is illustrated in Figure 1. The amount of nitrogen extracted increases as buffer concentration is raised. However, increasing buffer concentration results in a decrease in the rate of filtering the aqueous bean extract.

Figure 2 shows that the amount of nitrogen extracted and the filtration rate of the aqueous bean extract are related to the amount of enzyme added.

Increasing substrate concentration does not greatly affect the amount of nitrogen extracted (Figure 3). However, a progressive decrease in the filtration rate occurs as substrate concentration is increased. This is probably caused by the presence of a greater amount of gummy substances in the substrate.

The optimum pH of filtering the bean extract was found

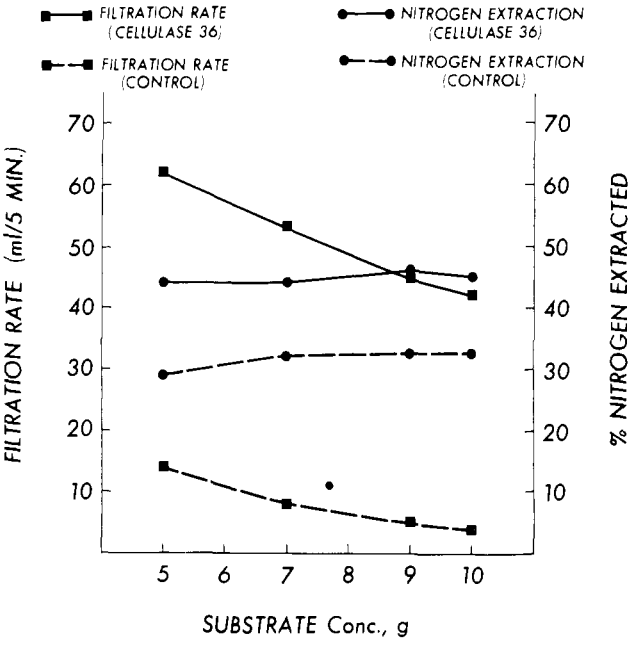

Figure 3. Effect of substrate concentration. Experimental procedure was the same as the standard method except that different substrate concentrations were used

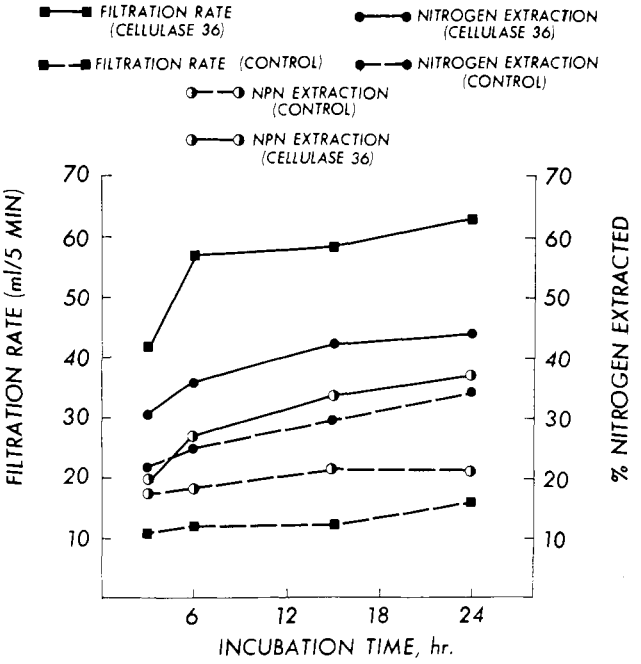

Figure 5. Effect of incubation time. Experimental procedure was the same as the standard method except that the reaction mixture was incubated for varying periods of time

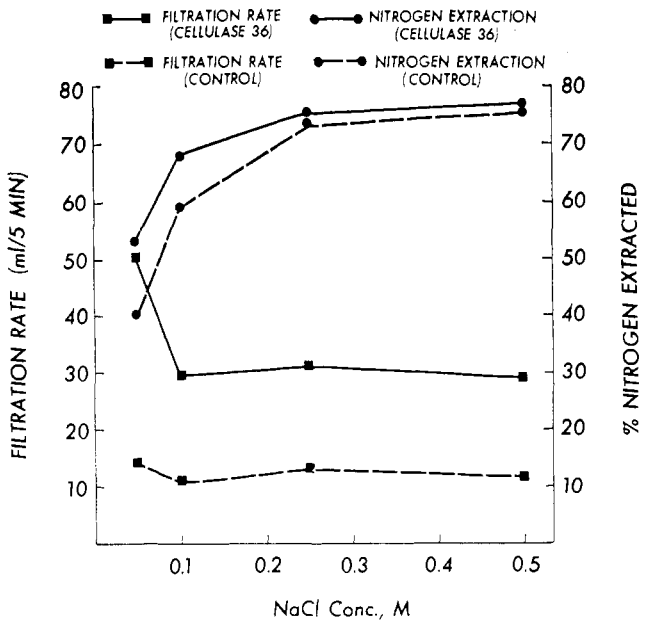

Figure 6. Effect of NaCl concentration. Experimental procedure was the same as the standard method except that 0.05*M* sodium acetate buffer containing different NaCl concentrations was used

to be pH 4.0, but nitrogen extraction was optimum at pH 5.6 (Figure 4).

A progressive increase in both nitrogen extraction and filtration rate occurs as the time of incubation is prolonged, as illustrated in Figure 5. The amount of nonprotein nitrogen is also progressively increased, confirming the earlier report that Cellulase 36 has proteolytic activity (Rohm and Haas Co., 1964).

The effect of NaCl concentration on the enzymatic improvement of nitrogen extraction and filtration rate is shown in Figure 6. A sharp decrease in the rate of filtering the aqueous bean extract occurs as NaCl concentration is raised. However, raising NaCl concentration results in a sharp increase in the amount of nitrogen extracted. Sodium chloride at higher concentrations was almost as effective in enhancing extractability as was the enzyme treatment.

Products of enzymatic hydrolysis of bean carbohydrates are given in Table I. The sugar content of pea beans as determined by hot 80% ethanol is high in stachyose, raffinose, and sucrose, but low in free sugars. However, large amounts of arabinose, galactose, glucose, and fructose are produced as a result of treating the beans with Cellulase 36. They are apparently the products of enzymatic hydrolysis of stachyose, raffinose, and sucrose. A few unidentified reducing oligosaccharides (2–4 monosaccharide units) are also present in smaller quantities. In the control, the disappearance of stachyose and raffinose resulted in the presence of a greater amount of sucrose, galactose, and fructose. This is obviously caused by the eznymes present in the beans. The data in Table I thus suggest that enzymes effectively remove stachyose, the major cause of flatulence in beans.

The data obtained in this work clearly indicate that Cellulase 36 is not only capable of enhancing extractability of the nitrogenous constituents from pea beans, but is also able to increase greatly the rate of filtering the aqueous bean extract. The improvement in the filterability of the bean extract apparently resulted from the enzymatic hydrolysis of the gummy substances present in the beans (Table I). Morimoto *et al.* (1962) also found that Cellulase 36 is fairly effective in degrading the gums from some tropical legumes.

Table I. Products of Enzymatic Hydrolysis of Bean Carbohydrates

	% Sugar, Dry Weight Basis		
	Extracted with hot 80% ethanol	Control[a]	Cellulase 36[a]
Stachyose	3.43	1.39	0.34
Raffinose	0.73	0.17	0.01
Sucrose	2.86	3.56	0.02
Galactose	0.06	1.11	1.74
Glucose	...	...	3.97
Fructose	...	0.25	1.29
Arabinose	...	...	2.01

[a] Experimental procedure was the same as the standard method.

The use of microbial enzymes in enhancing the extractability of the nitrogenous constituents from oilseeds and pulses has been reported. Yasumatsu *et al.* (1966) solubilized approximately 78% of the nitrogenous constituents of soybean meal with the crude enzymes of *Trametes sanguinea*, which has the activities of acid protease, cellulase, and glucanase. Abdo and King (1967) found that the enzymes produced by *Pestalotiopsis westerdijkii* are capable of solubilizing 96% of the nitrogenous constituents of soybeans. The enzyme preparation was reported to contain a variety of carbohydrases, proteases, lipases, and even some oxidases. Sreekantiah *et al.* (1969) recently showed that the commercial enzyme preparation obtained from *Trametes sanguinea* solubilized 64 and 86% of the nitrogenous constituents of sesame meal and chickpea, respectively. Obviously from the results obtained in this work, Cellulase 36, a food grade enzyme preparation derived from *Aspergillus niger*, is capable of greatly enhancing the extractability of the nitrogenous constituents from pea beans and the exact amount of nitrogen extracted is dependent upon the conditions used. The increase in the amount of nitrogen extracted appeared to be a result of enzymatic hydrolysis of both bean proteins and carbohydrates. This observation thus demonstrates that the use of Cellulase 36 to enhance the extraction of nitrogenous constituents from pea beans and improve the filterability of the aqueous bean extract is a technically feasible process. The results shown in this report should be of practical interest to the food industry.

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