Studies on the Extraction of Proteins from Red Kidney Beans. I. Improvement in Filterability of Aqueous Bean Extract[1]

Y. D. HANG, W. F. WILKENS, A. S. HILL, K. H. STEINKRAUS, and L. R. HACKLER, Cornell University, New York State Agricultural Experiment Station, Geneva, New York

ABSTRACT

It has been observed that the aqueous extract of red kidney beans was extremely difficult to filter. Attempts have been made to eliminate the difficulty in filtration of the bean extract through the use of some commercial food-grade enzyme preparations. Among the enzymes used, only Cellulase 36 and Rhozyme HP-150 were found satisfactory for this purpose. The improvement in filterability of the bean extract appeared to be a result of enzymatic breakdown of bean polysaccharides and oligosaccharides into simple, soluble sugar units. The enzymes were also capable of improving greatly the extractability of nitrogenous constituents from red kidney beans.

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As population increases, there is a greater need for new sources of protein of high nutritive value with acceptable flavor and texture for formulation of synthetic and new types of food. Although there is a considerable background of information regarding the extraction and processing of soybean proteins and soy-based foods (1,2,3), there is very little information in the literature dealing with practical methods of extracting proteins from red kidney beans (*Phaseolus vulgaris*). Soybeans are rich in protein and fat but contain little starch, whereas red kidney beans have a lower protein and fat content than soybeans and a higher concentration of starch. It is necessary to separate the starch to prepare a protein-rich fraction. Thus, there is a need for development of methods of separating the starch and protein fractions of red kidney beans.

Results obtained in our earlier extraction work (unpublished data) indicate that the aqueous extract of red kidney beans is extremely difficult to filter, probably because of the presence of gummy substances in the seeds. There is thus a need for methods that will eliminate the gummy substances and, as a result, improve the rate of filtering the bean extract. Several commercial food-grade enzyme preparations, such as Cellulase 36, Klerzyme 200, and Pectinol 10M, have been found to degrade the gums from some tropical legumes (4). Rhozyme HP-150, a food-grade enzyme preparation, has also been reported to cause a 94.2% decline in the viscosity of a 0.2% locust bean gum solution at pH 4.0 and 40°C. during a reaction time of 2 hr. Abdo and King (5) have found that enzymes derived from *Pestalotiopsis westerdijkii* are capable of facilitating the extraction of soybean proteins. The resulting protein extracts appeared to be free of toxic products and to have a nutritive value equivalent to that of extracts prepared without enzyme treatment. They have also reported that the enzyme preparations are complex, including a variety of carbohydrases, proteases, lipases, and even some oxidases. It remains uncertain which of these enzymes are the cause of the added extractability of soybean proteins and which merely occur concurrently. This paper describes the conditions whereby commercial food-grade enzyme preparations can improve the filterability of an aqueous extract of red kidney beans. Data on the enzymatic improvement in extractability of nitrogenous constituents from red kidney beans are also included.

MATERIALS AND METHODS

For these investigations, certified red kidney beans were purchased from Agway, Inc. (Geneva, N.Y.). The seeds were ground through the 1B screen (13 holes per linear in.) of a Fitz mill, Model D (W. J. Fitzpatrick Co., Chicago, Ill.).

Sources of commercial enzyme preparations used in this work were as follows: Cellulase, Rhozyme HP-150, and Pectinol 10M, Rohm and Haas Co.; Klerzyme 200, Wallerstein Co.; malt-diastase, Mann Research Lab. These enzyme preparations generally contain a number of secondary enzyme systems.

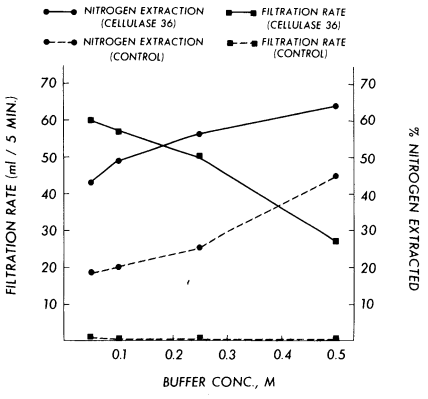
The standard extraction procedure involved the blending of 5 g. of ground beans with 100 ml. of 0.1M sodium acetate buffer (pH 4.0) in a Waring Blendor for 3 min. To this mixture 100 mg. of enzyme and 1 ml. of toluene were added. The contents were then thoroughly mixed and incubated at 43°C. for 22 hr. 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

TABLE I. EFFECT OF ENZYMES ON THE FILTRATION RATE AND NITROGEN EXTRACTION[a]

Enzyme	Filtration Rate ml./5 min.	Total N Extracted %	Nonprotein N Extracted %
Control	0.5	20.4	19.4
Cellulase 36	57.1	49.0	29.7
Rhozyme HP-150	52.7	36.2	34.5
Pectinol 10M	3.7	27.9	24.2
Klerzyme 200	6.9	43.2	39.9
Malt-diastase	0.8	42.1	20.3

[a]Experimental procedure was the same as standard method except that different enzymes were used.

No. 2v folded filter paper in a 100-mm. funnel. Under the same conditions, the filtration rate of 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 (6), replacing mercuric oxide and potassium sulfate with a Kjeldahl tablet containing sodium sulfate and selenium as the catalyst (British Drug Houses, Ltd., Poole, England). Nonprotein nitrogen was determined by the method of Becker et al. (7). Sugar determination was carried out as described by Shallenberger and Moores (8). A unidirectional descending technique was used for separation of the sugars. Paper chromatograms were developed for 18 hr., or as long as needed to effect good separation of the sugars present. Chromatographic solvents were butanol:acetic acid:water (4:1:5) and butanol:ethanol:water (40:11:19).



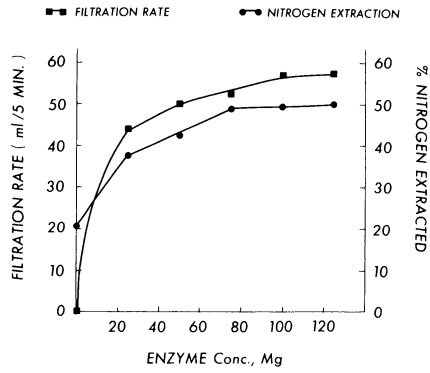


Fig. 1 (left). Effect of buffer concentration. Experimental procedure was the same as standard method except that different buffer concentrations were used.

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

RESULTS

Effect of Enzymes on Filtration Rate and Nitrogen Extraction

The results (Table I) indicate that the faster rate of filtering the bean extract is obtained with Cellulase 36 or Rhozyme HP-150. Klerzyme 200 and Pectinol 10M only increase the filtration rate slightly; malt-diastase is much less effective. The amount of nitrogen extracted is also greatly increased as a result of enzyme treatment. All the enzyme preparations used in this work have been found to possess proteolytic activity.

Effect of Buffer Concentration

It appears that the rate of filtering the bean extract decreases as buffer concentration is increased (Fig. 1). However, the amount of nitrogen extracted greatly increases as buffer concentration is raised.

Effect of Enzyme Concentration

The initial filtration rate and the amount of nitrogen extracted are proportional to the amount of enzyme added. Both filtration rate and nitrogen extraction gradually reach maxima as enzyme concentration is further increased (Fig. 2).

Effect of Substrate Concentration

A progressive decrease in the filtration rate occurs as substrate concentration is increased (Fig. 3). This is probably caused by increasing amounts of gummy substances in the substrate. However, the amount of nitrogen extracted is only slightly affected by increasing substrate concentration.

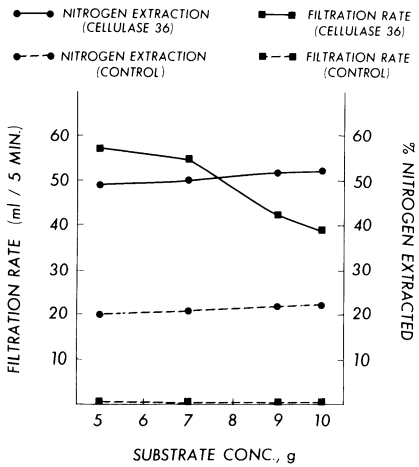


Fig. 3 (left). Effect of substrate concentration. Experimental procedure was the same as standard method except that different substrate concentrations were used.

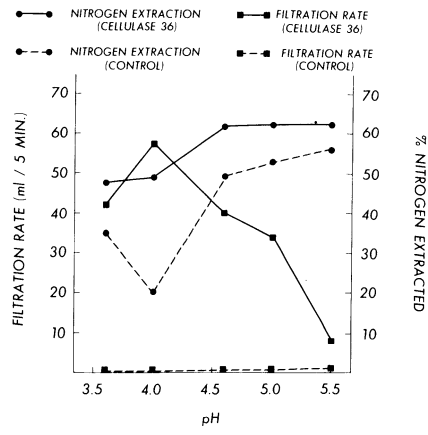
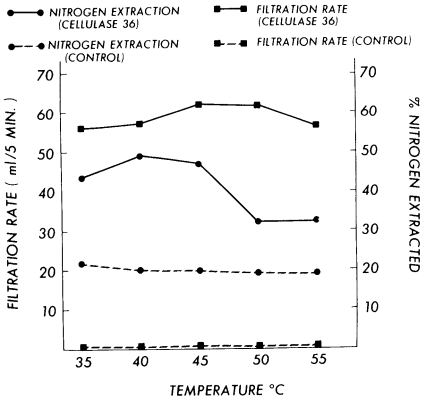


Fig. 4 (right). Effect of pH. Experimental procedure was the same as standard method except that 0.1M sodium acetate buffer at different pH was used.



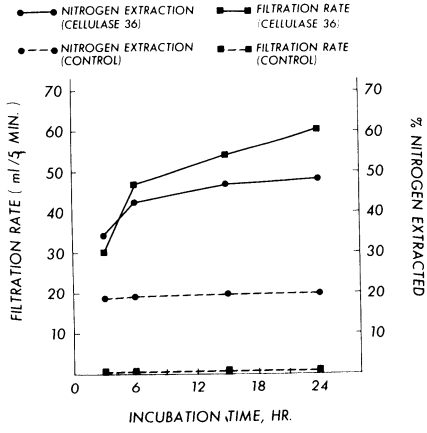


Fig. 5 (left). Effect of temperature. Experimental procedure was the same as standard method except that reaction mixture was incubated at different temperatures for 15 hr.

Fig. 6 (right). Effect of incubation time. Experimental procedure was the same as standard method except that reaction mixture was incubated for various periods of time.

Effect of pH

The optimum pH for enzymatically improving the filtration rate and nitrogen extraction is pH 4.0 (Fig. 4).

Effect of Temperature

Enzymatic improvement in the filtration rate of red kidney bean slurries is optimum in the range of 45° to 50°C.; however, nitrogen extraction is markedly decreased at 50°C. The optimum temperature for nitrogen extraction is 40°C. (Fig. 5).

Effect of Incubation Time

The addition of enzyme causes a progressive increase in both filtration rate and nitrogen extraction as the time of incubation is prolonged (Fig. 6).

Effect of NaCl Concentration

The amount of nitrogen extracted greatly increases as NaCl concentration is raised (Fig. 7). However, the rate of filtering the bean extract is not greatly affected by increasing NaCl concentration. It is concluded that salt is not necessary when treating with enzymes.

Products of Enzymatic Degradation of Bean Polysaccharides

Large amounts of glucose, galactose, fructose, xylose, and arabinose are produced as a result of the enzymatic hydrolysis of bean polysaccharides and oligosaccharides (Table II). A few reducing oligosaccharides (2 to 4 monosaccharide units), which are as yet unidentified, are produced in smaller quantities in this hydrolysis.

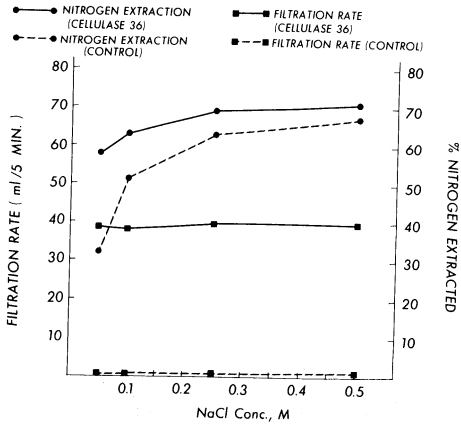


Fig. 7. Effect of NaCl concentration. Experimental procedure was the same as standard method except that 0.05M sodium acetate buffer (pH 4.0) containing different NaCl concentrations was used.

DISCUSSION

The data shown in the present work clearly indicate that the difficulty in filtration of the bean extract can be successfully eliminated through the use of some commercial food-grade enzyme preparations. Among the enzymes used, only Cellulase 36 and Rhozyme HP-150 were found satisfactory for this purpose. The improvement in the filterability of the bean extract appeared to be a result of the enzymatic breakdown of bean polysaccharides and oligosaccharides into simple, soluble monosaccharide units (Table II). Degradation of the gums from some legumes by enzymes has also been used with some success. Moromoto et al. (4) found that Cellulase 36 was fairly effective in degrading the gums from a number of tropical legumes to monosaccharide units, whereas Klerzyme 200 and Pectinol 10M were less effective.

TABLE II. PRODUCTS OF ENZYMATIC DEGRADATION OF BEAN CARBOHYDRATES

	Sugar, Dry Basis			
	Extracted with Hot 80% Ethanol %	Control[a] %	Cellulase 36[a] %	Rhozyme HP-150[a] %
Stachyose	3.72	2.62	1.39	1.97
Raffinose	0.61	0.24	0.13	...
Sucrose	2.95	3.29	0.16	0.05
Galactose	0.02	1.33	2.18	1.75
Glucose	...	0.69	4.95	9.88
Fructose	...	0.96	1.38	4.03
Xylose	...	...	...	1.29
Arabinose	...	...	1.89	4.44

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

Enzyme preparations used in this work not only are capable of eliminating the filtration barrier, but also are able to improve greatly the extractability of nitrogenous constituents from red kidney beans. Cellulase 36 appears to be the most promising of the enzymes investigated (Table I). Solubilization of soybean proteins by the enzymes derived from microorganisms has been reported by Abdo and King (5). Cellulase 36 used in the present investigation is a food-grade enzyme preparation of microbiological origin and exhibits hydrolytic activity on a variety of substrates including cellulose, cellulose derivatives, pectin, protein, pentosans, and hexosans. It is probable that the improvement in the extractability of nitrogenous constituents from red kidney beans is the result of the enzymatic hydrolysis of both bean carbohydrates (Table II) and proteins (Table I). The present report thus demonstrates that improvement in both the rate of filtration and the extraction of nitrogenous constituents from red kidney beans through the use of commercial food-grade enzyme preparations is technically feasible. The data presented in this paper should be of interest to the food industry.

Acknowledgment

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