Enzymatic Solubilization of Nitrogenous Constituents of Mung Beans

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Cellulase 36, a food grade enzyme preparation derived from *Aspergillus niger*, was found to be capable of facilitating the solubilization of the nitrogenous constituents of mung beans. The increase in the amount of nitrogen solubilized appeared to be a result of the enzymatic hydrolysis of both bean proteins and carbohydrates. The effect of various factors such as sodium acetate buffer concentration, enzyme concentration, substrate concentration, pH, temperature, incubation time, and NaCl concentration on the enzymatic solubilization of the nitrogenous constituents of mung beans was investigated.

Mung beans are cultivated throughout the southern part of Asia, in the Malay Islands, and quite generally throughout the eastern portion of Africa. They have also been grown with some success in the midwest and southern regions of the United States. The seeds are used in India almost exclusively as human foods, and the straw is fed to cattle (Johns and Waterman, 1920). In China, a string-like product called Fentiao, somewhat resembling spaghetti in physical appearance, is made from mung beans. Fentiao is used in soups or mixed with vegetables that are prepared by boiling (Smith, 1961).

Yasumatsu *et al.* (1966) found that the enzymes produced by *Trametes sanguinea* are capable of solubilizing soybean meal and yeast cells efficiently. Abdo and King (1967) reported more efficient extraction of protein from soybeans treated with the enzymes derived from *Pestalotiopsis westerdijkii*. The present study was undertaken to evaluate the capability of Cellulase 36, a food grade enzyme preparation derived from *Aspergillus niger* (Faith, 1969), to solubilize the nitrogenous constituents of mung beans.

EXPERIMENTAL

Certified mung beans of the common, olive-green variety used throughout this work were kindly supplied by the Specialty Food Corporation (Johnson City, 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 Company, Chicago, Ill.).

Cellulase 36 used in this study was kindly provided by Rohm and Haas Company, Philadelphia, Pa.

Total nitrogen was analyzed by a slight modification of the standard micro-Kjeldahl method (A.O.A.C., 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). Sugar was estimated according to the method of Shallenberger and Moores (1957), and the procedure of Becker *et al.* (1940) was used for determination of nonprotein nitrogen.

RESULTS AND DISCUSSION

In assessing the effect of sodium acetate buffer concentration on the enzymatic solubilization of the nitrogenous constituents of mung beans, 5 grams of ground beans were blended with 100 ml. of sodium acetate buffer (pH 4.0) at varying concentrations in a Waring Blendor for 3 minutes. 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 hours. After filtration through Whatman No. 2V folded filter, micro-Kjeldahl analyses were run on the filtrates. Controls without enzyme were prepared in exactly the same manner. The results are shown in Figure 1. The nitrogen content of enzyme has been substracted from that of the appropriate extracts. The amount of nitrogen solubilized increases as sodium acetate buffer concentration is raised.

In determining the influence of enzyme concentration on the amount of nitrogen solubilized, 5 grams of ground beans were blended with 100 ml. of 0.1*M* sodium acetate buffer (pH 4.0) in a Waring Blendor for 3 minutes. To this mixture varying amounts of enzyme and 1 ml. of toluene were added. The contents were then thoroughly mixed and incubated at 43° C for 22 hours. After filtration through Whatman No. 2V folded filter paper, micro-Kjeldahl analyses were run on the filtrates. Controls without enzyme were prepared in exactly the same manner. The results are presented in

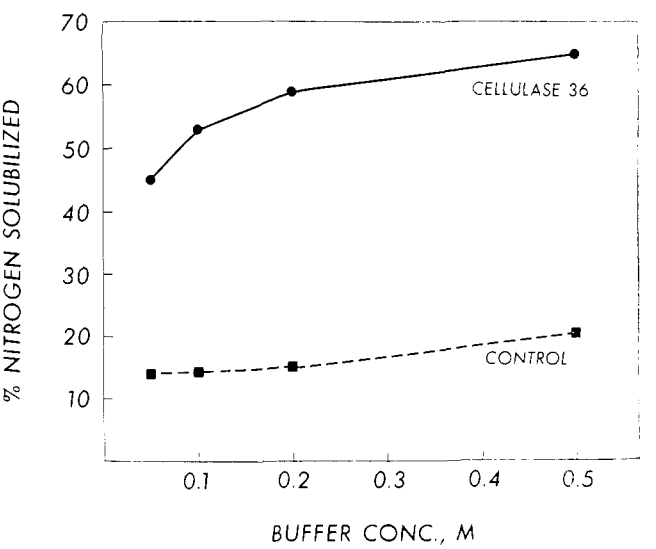

Figure 1. Effect of sodium acetate buffer concentration

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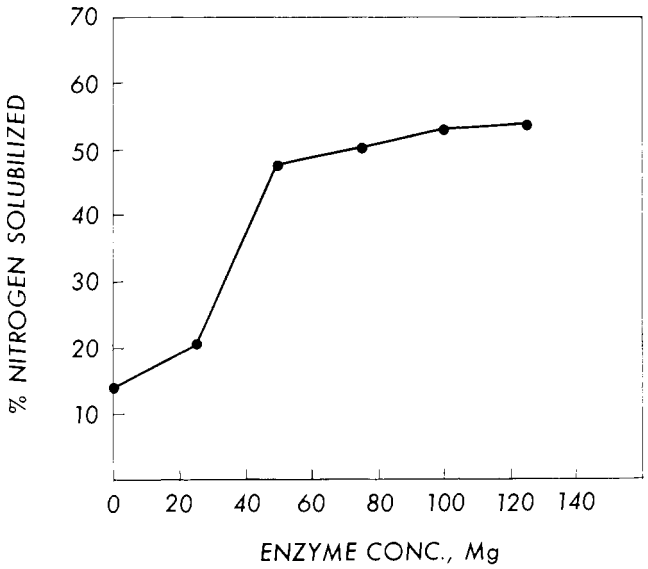

Figure 2. Effect of enzyme concentration

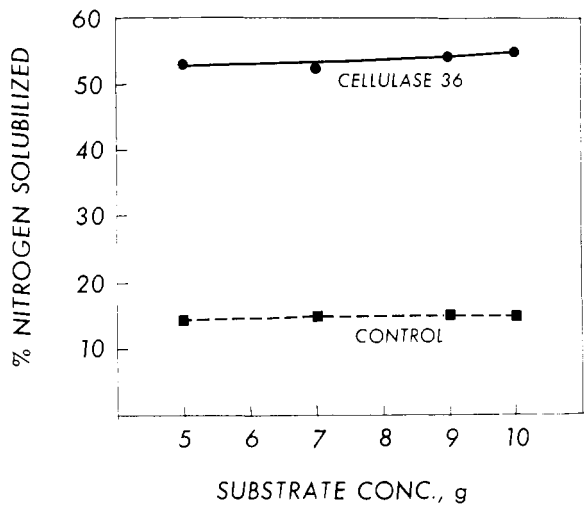

Figure 3. Effect of substrate concentration

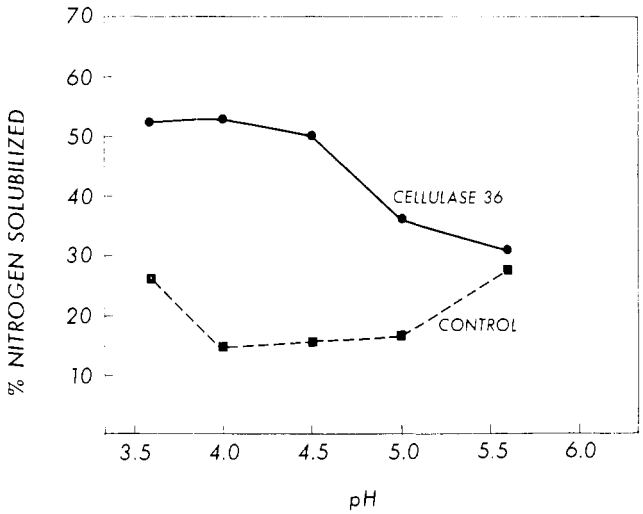

Figure 4. Effect of pH

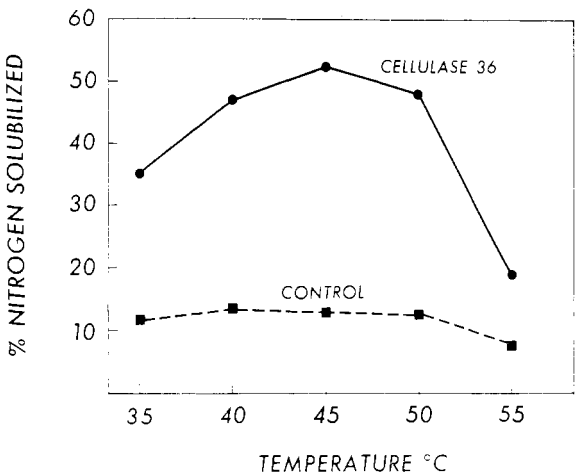

Figure 5. Effect of temperature

Figure 2. The nitrogen content of enzyme has been substracted from that of the appropriate extracts. The amount of nitrogen solubilized initially appears to be related to the amount of enzyme added. This relationship eventually decreases as enzyme concentration is further increased.

In studying the effects of substrate concentration on the enzymatic solubilization of the nitrogenous constituents of mung beans, varying amounts of ground beans were blended with 100 ml. of 0.1*M* sodium acetate buffer (pH 4.0) in a Waring Blendor for 3 minutes. 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 hours. After filtration through Whatman No. 2V folded filter paper, micro-Kjeldahl analyses were run on the filtrates. Controls without enzyme were prepared in exactly the same manner. The results shown in Figure 3 indicate that the amount of nitrogen solubilized is only slightly affected by increasing substrate concentration. The nitrogen content of enzyme has been substracted from that of the appropriate extracts.

In establishing the effect of pH on the enzymatic solubilization of the nitrogenous constituents of mung beans, 5 grams of ground beans were blended with 100 ml. of 0.1*M* sodium acetate buffer at desired pH values in a Waring Blendor for 3 minutes. 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 hours. After filtration through Whatman No. 2V folded filter paper, micro-Kjeldahl analyses were run on the filtrates. Controls without enzyme were prepared in exactly the same manner. The optimum pH for enzymatically solubilizing the nitrogenous constituents of mung beans was found to be pH 4.0 (Figure 4). The nitrogen content of enzyme has been substracted from that of the appropriate extracts.

In assessing the influence of temperature on the enzymatic solubilization of the nitrogenous constituents of mung beans, 5 grams of ground beans were blended with 100 ml. of 0.1*M* sodium acetate buffer (pH 4.0) in a Waring Blendor for 3 minutes. To this mixture 100 mg. of enzyme and 1 ml. of toluene were added. The contents were then thoroughly mixed and incubated at desired temperatures for 15 hours. After filtration through Whatman No. 2V folded filter paper, micro-Kjeldahl analyses were run on the filtrates. Controls without enzyme were prepared in exactly the same manner. The optimum temperature for enzymatically solubilizing the nitrogenous constituents of mung beans was found to be 45° C (Figure 5). The nitrogen content of enzyme has been substracted from that of the appropriate extracts.

In determining the effect of incubation time on the enzymatic solubilization of the nitrogenous constituents of mung

beans, 5 grams of ground beans were blended with 100 ml. of 0.1*M* sodium acetate buffer (pH 4.0) in a Waring Blendor for 3 minutes. 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 varying periods of time. After filtration through Whatman No. 2V folded filter paper, micro-Kjeldahl analyses were run on the filtrates. Controls without enzyme were prepared in exactly the same manner. Progressive increases occur in both the amount of total nitrogen and that of nonprotein nitrogen solubilized as the time of incubation is prolonged (Figure 6). The nitrogen content of enzyme has been substracted from that of the appropriate extracts. These data thus confirm the earlier report that Cellulase 36 possesses proteolytic activity (Rohm and Haas Company, 1964).

In establishing the influence of NaCl concentration on the enzymatic solubilization of the nitrogenous constituents of mung beans, 5 grams of ground beans were blended with 100 ml. of 0.05*M* sodium acetate buffer (pH 4.0) containing varying amounts of NaCl in a Waring Blendor for 3 minutes. 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 hours. After filtration through Whatman No. 2V folded filter paper, micro-Kjeldahl analyses were run on the filtrates. Controls without enzyme were prepared in exactly the same manner. The results are shown in Figure 7. The nitrogen content of enzyme has been substracted from that of the appropriate extracts. Apparently, increasing NaCl concentration results in a sharp increase in the amount of nitrogen solubilized. Higher concentrations of NaCl were almost as effective in enhancing extractability as was the enzyme treatment.

The products of enzymatic hydrolysis of bean carbohydrates are given in Table I. As a result of enzyme treatment, large amounts of glucose, galactose, and fructose are produced. A few reducing oligosaccharides (2 to 4 monosaccharide units), which are not yet identified, are also produced in smaller quantities. This is apparently caused by the enzymatic hydrolysis of bean polysaccharides and oligosaccharides. Small amounts of glucose, galactose, and fructose are also present in the control. They are probably the products of the degradation of stachyose, raffinose, and sucrose by the enzymes present in mung beans.

Table I. Products of Enzymatic Hydrolysis of Bean Carbohydrates

	% Sugar, Dry Weight Basis		
	Extracted with hot 80% ethanol	Control[a]	Cellulase 36[a]
Stachyose	1.87	1.82	0.58
Raffinose	0.43	0.29	0.30
Sucrose	1.27	1.01	0.14
Galactose	0.04	0.93	0.94
Glucose	trace	0.64	4.30
Fructose	trace	0.50	1.20
Arabinose	...	...	1.90
Total sugars	3.61	5.19	9.36

[a] Five grams of ground beans were blended 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 for 43° C for 22 hrs. After filtration through Whatman No. 2V folded filter paper, sugar analyses were run on the filtrates. Control without enzyme was prepared in exactly the same manner.

The results presented in this report thus give evidence that Cellulase 36 is capable of facilitating the solubilization of the nitrogenous constituents of mung beans. The use of enzymes derived from microorganisms in the solubilization of soybean proteins has been reported. Yasumatsu *et al.* (1966) found that enzymes produced by *Trametes sanguinea* are very effective in solubilizing soybean meal and yeast cells. Their data revealed the presence of proteases, cellulases, and glucanases in the enzyme preparation. Abdo and King (1967) also demonstrated that enzymes derived from *Pestalotiopsis westerdijkii* are capable of improving greatly the extraction of soybean proteins. Their enzyme preparation was reported to be a complex one including a variety of carbohydrases, proteases, lipases, and even some oxidases. Which of these enzymes are the cause of the added extractability of soybean proteins remains uncertain. Cellulase 36 (Rohm and Haas Company, 1964) used in this work is a food grade enzyme preparation derived from *Aspergillus niger* and exhibits hydrolytic activity on a variety of substrates, including cellulose, cellulose derivatives, pectin, protein, pentosans, and hexosans. Obviously from the data obtained in this study,

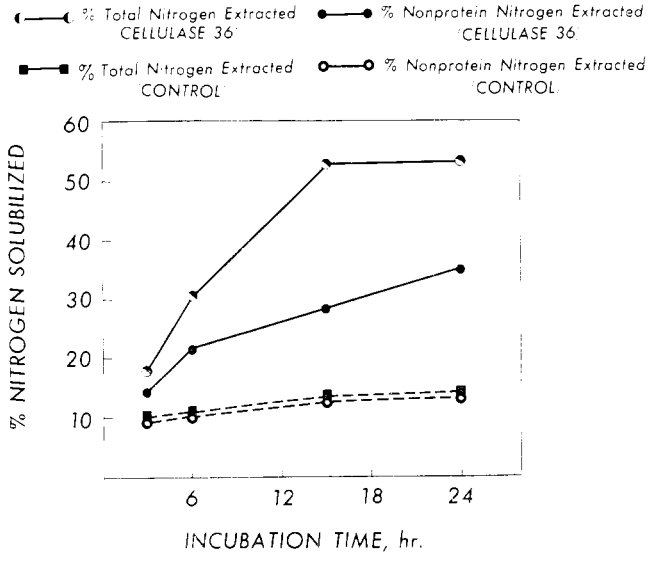

Figure 6. Effect of incubation time

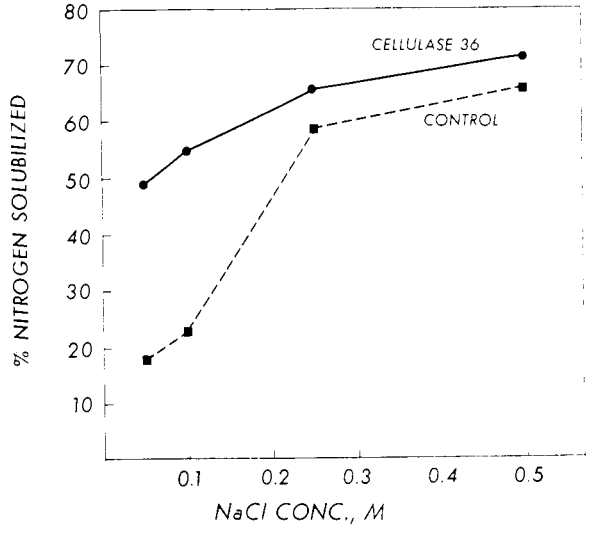

Figure 7. Effect of NaCl concentration

the increase in the amount of nitrogen solubilized is a result of the enzymatic hydrolysis of both bean proteins and carbohydrates.

Enzymes derived from *Trametes sanguinea* were capable of solubilizing 78% of the nitrogenous constituents in soybean meal (Yasumatsu *et al.*, 1966). Abdo and King (1967) have solubilized 96% of soybean protein with enzyme produced from *Pestalotiopsis westerdijkii*. Cellulase 36, a food grade enzyme preparation derived from *Aspergillus niger*, also greatly facilitated the solubilization of the nitrogenous constituents of mung beans and the exact amount of nitrogen solubilized is dependent upon the conditions used. These observations thus demonstrate that the use of microbial enzymes to solubilize the proteins of plant origin is a feasible process, and the results shown in this report might be of interest to many people engaged in increasing the use of vegetable proteins for foods.

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