Improvement in Soluble Fiber Content of Wheat Fiber through Enzymatic Modification

Young Nam Oh and Melvin L. Grundleger*

Department of Food Science and Human Nutrition, Colorado State University, Ft. Collins, Colorado 80523

The influence of fiber purification and conditions of enzymatic modification on soluble fiber content of wheat fiber were examined. Crude wheat fiber was purified by a modified enzymatic extraction. Crude wheat bran contained 16% soluble dietary fiber by weight. Purification of the crude wheat bran did not alter soluble fiber content nor result in a significant loss of total dietary fiber. A technical grade hemicellulase was used to enzymatically modify the purified wheat fiber. The yield varied considerably with enzyme concentration and time. The optimized modification conditions utilized were 0.1 M acetate buffer (pH 5.0) and 60 HCU/mL per g of PIFF hemicellulase at 30 °C for 40 min. The enzymatic modification of wheat fiber resulted in an 87% increase in soluble fiber content up to 30%.

A number of studies have demonstrated that the use of soluble fibers may be an important adjunct in the treatment of diabetes. Jenkins et al. (Jenkins et al., 1977a,b, 1980) demonstrated that the addition of soluble types of dietary fiber such as pectin and guar into test meals reduced postprandial glycemia in normal, non-insulin-dependent and insulin-dependent subjects. This was attributed in part either to an increased mouth to cecum transit time, to the decreased rate of gastric emptying, or to a slower rate of duodenal absorption, all factors associated with an increase in solubility and/or viscosity (Blackburn et al., 1984; Jenkins et al., 1977b, 1979). Insoluble fibers such as cellulose or less soluble fibers such as wheat bran have been shown to be less effective as agents for lowering serum glucose values. In contrast, they have been shown to increase fecal bulk and inhibit colon tumors. Although wheat bran is a less soluble type of fiber, a number of studies have reported the use in the treatment of diabetics (Simpson et al., 1979a,b).

The objective of the present investigation was to determine the conditions under which the soluble fiber content of wheat fiber could be increased. This would increase the potential for clinical usage under circumstances requiring a more soluble type of fiber.

PROCEDURES

Purification of Wheat Bran. A modification of Prosky et al. (1984) and Monte and Maga (1980) was utilized for determination and purification of a commercial grade of crude wheat bran. The purification method developed is described in Figure 1.

Sample Pretreatment-Extraction of Lipids. The extraction of lipid from crude wheat bran (CWB) was performed three times with use of reagent grade petroleum ether (PET) (6:1 = PET:CWB) at room temperature. The defatted wheat bran (DWB) was dried overnight at 105 °C in an air oven to a constant weight. It was then passed through a Wiley mill (Model No. 4) equipped with a 0.5-mm-mesh screen.

Removal of Soluble Complex Carbohydrate. DWB was incubated in a 0.08 M Na_2PO_4 buffer (pH 6.0 DWB), containing Termamyl 120 L (120 KNU (Novo-α-amylase unit)/mL per 10 g of DWB; heat-stable α-amylase; Novo Laboratories, Inc., Wilton, CT 06897) for 30 min in a hemispherical heating mantle. The incubation period started when the contents reached 85–95 °C. After the incubation period, the sample was removed from the hemispherical heating mantle, cooled to room temperature (≈20 °C), and adjusted to pH 7.5.

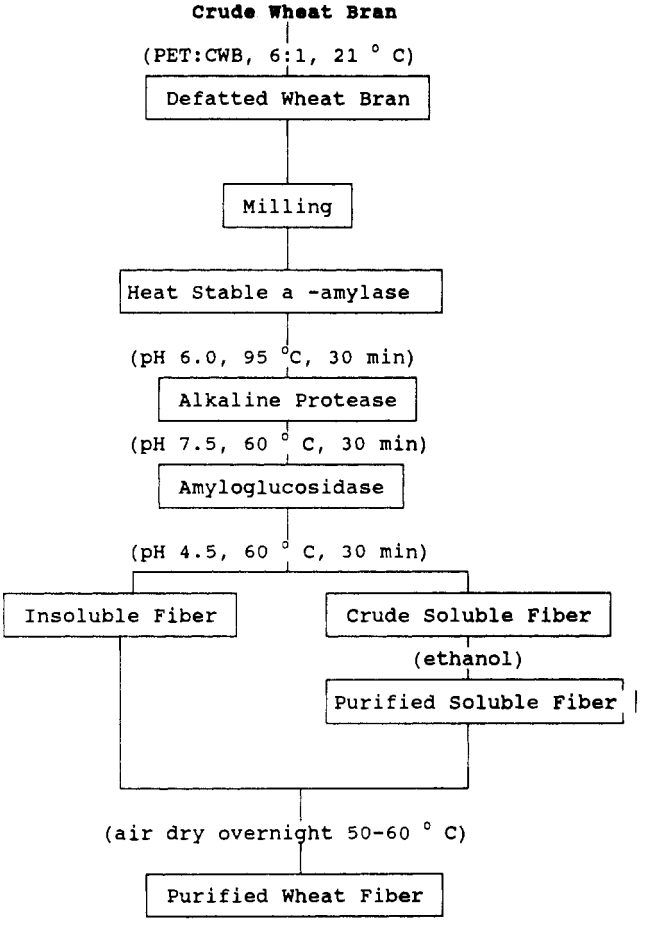

Figure 1. Flow sheet representation of enzymatic purification.

Removal of Water-Soluble Proteins. After pH adjustment, an alkaline protease (2.4 Anson unit (AU)/mL per 10 g of DWB; Alcalase 2.4 L; Novo Laboratories) was added to the incubate. The samples were again incubated in a hemispherical heating mantle at 60 °C for 30 min with constant stirring. The incubation period began when the contents reached 60 °C. The contents were then cooled to room temperature, and the pH was adjusted to 4.5.

0021-8561/90/1438-1142$02.50/0

Removal of Insoluble Complex Carbohydrates. Removal of the carbohydrate was accomplished by the addition of amyloglucosidase (AMG) (200 amyloglucosidase unit (AGU)/mL per 10 g of DWB; AMG 200 L; Novo Laboratories). The samples were incubated at 55–65 °C for 30 min in a hemispherical heating mantle with constant stirring.

Separation of Soluble and Insoluble Fiber Fractions. After enzyme treatment, the insoluble fiber fraction was obtained by sedimentation of samples at 4000*g* for 10 min in polycarbonate centrifuge bottles. The soluble fiber fraction was obtained by adding four volumes of 95% ethanol to the supernatant. The resulting precipitate was then centrifuged at 4000*g* for 10 min with use of polycarbonate centrifuge bottles to sediment the soluble fiber fraction. Then insoluble and soluble fiber fraction were mixed together and dried overnight at 50–60 °C in an air oven until constant weight was achieved. Total dietary fiber in each sample was equal to the combined fractions.

Modification of Purified Wheat Bran. The modification of the purified wheat bran consisted of a purification procedure as described above followed by the enzymatic treatment of the purified insoluble fiber fraction. Considering that the major fiber constituent of wheat bran is hemicellulose, we chose a hemicellulase (Gammanase 1.5 L; Novo Laboratories), which produced a partial degradation of the wheat fiber hemicellulose necessary for modification of the insoluble fiber fraction. Preliminary experiment using Pectinex and Pectinex Ultra SP did not produce any change in the soluble fiber content of the purified fraction. Temperature was held constant at 30 °C since elevations to 37 and 50 °C resulted in a rapid hydrolysis of both soluble and insoluble fiber components. This was verified by a lack of alcohol-precipitable fiber. Optimal conditions for modification of the purified wheat bran were obtained by varying time of incubation and concentration of the hemicellulase used for the modification. Samples of the purified insoluble fiber fraction were incubated in a pH 5.0 acetate buffer under the conditions specified. Hemicellulase activity was calibrated against a standard preparation (Gammanase 1.5 L, Lot No. CHN-0004; Novo Laboratories). At the end of the incubation period, the insoluble fiber fraction was separated by centrifugation at 4000*g* for 10 min. Soluble fiber was obtained by adding 4 volumes of 95% ethanol and centrifuging at 4000*g* for 10 min to sediment the soluble fiber fraction. Separated insoluble fiber and soluble fiber fractions were mixed well and dried overnight at 50–60 °C until a constant weight was achieved. The modification method for purified wheat fiber is shown in Figure 2.

Effectiveness of Purification. Completeness of the lipid extraction was verified by extraction of fractions with a modified Folch procedure (Folch et al., 1957). Samples were extracted with CCl_4–MeOH (2:1, v/v) and evaporated to dryness under nitrogen. The completeness of removal of both soluble and insoluble carbohydrates was determined by enzymatic determination of glucose in an aliquot of the purified and modified fraction. Two methods were used for protein determination. The Kjeldahl method was the method of choice for nitrogen with a factor of 6.25 to convert percent nitrogen to protein. Similar results were obtained with use of a Bio-Rad micromethod for protein (Bradford, 1976). Proximate analysis of the crude wheat bran is shown in Table I.

Statistical Analysis. All fiber measurements are expressed as the means ± SE of three to five individual determinations. Differences between means were evaluated by the General Linear Models procedure of the Statistical Analysis System (SAS). The 3-dimensional plot was generated with the G3GRID procedure to produce a smoothed spline.

RESULTS AND DISCUSSION

In the present investigation conditions were established under which the soluble fiber content of wheat fiber was increased. The reported dietary fiber content of the commercial crude wheat bran in this study (51.5 ± 3.6% dry weight) was comparable to values reported by Demigne and Remesy (1985) and Van Soest et al. (1978) and somewhat higher than values reported by Southgate (1981). The crude wheat bran, purified by a modification of the methods described by Prosky et al. (1984) and Monte and Maga (1980), resulted in an increased recovery of both insoluble and soluble fiber fractions. The resulting purified insoluble fiber fraction (PIFF) was then subject to enzymatic modification under the conditions described above. The development of this method was such that it was used for preparation of purified fiber for use in feeding trials (Grundleger and Oh, 1989). Since preliminary investigations suggested that elevation of the reaction temperature resulted in a rapid loss of fiber, temperature was therefore held constant at 30 °C. An optimal hemicellulase concentration and time of incubation were achieved at 60 hemicellulase units (HCU)/mL per g of PIFF) and 40 min, respectively. The activity of the enzyme under the present conditions was such that we obtained a sharp peak of solubilization, which fell off rapidly above or below the 60 HCU/mL per g of PIFF. This has made the production of the modified wheat fiber (MWF) a fairly simple task (Grundleger and Oh, 1989). Preliminary studies showed that the use of a commercial enzyme preparations such as Pectinex or Pectinex Ultra SP-L, although containing a significant amount of polygalacturonase activity, was unsuitable for modification of the purified wheat fiber. The distribution of dietary fiber found in three fiber samples (CWF, PWF, MWF)

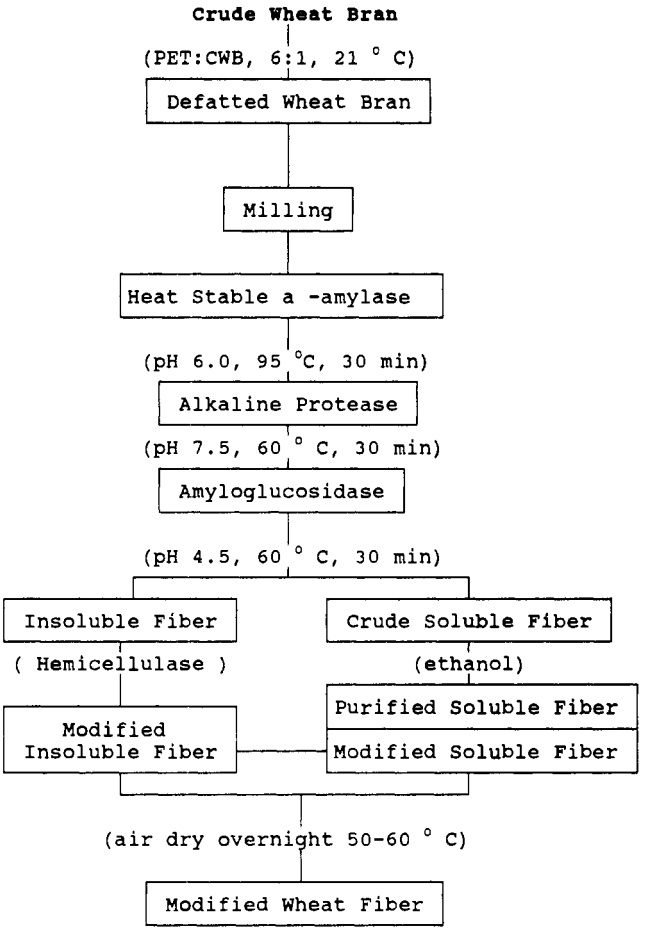

Figure 2. Flow sheet representation of enzymatic modification.

Table I. Proximate Analysis of Crude Wheat Bran

	% wheat bran
protein	9.4
lipid	5.7
carbohydrate	17.5
ash	5.8
water	10.1
crude fiber	51.5

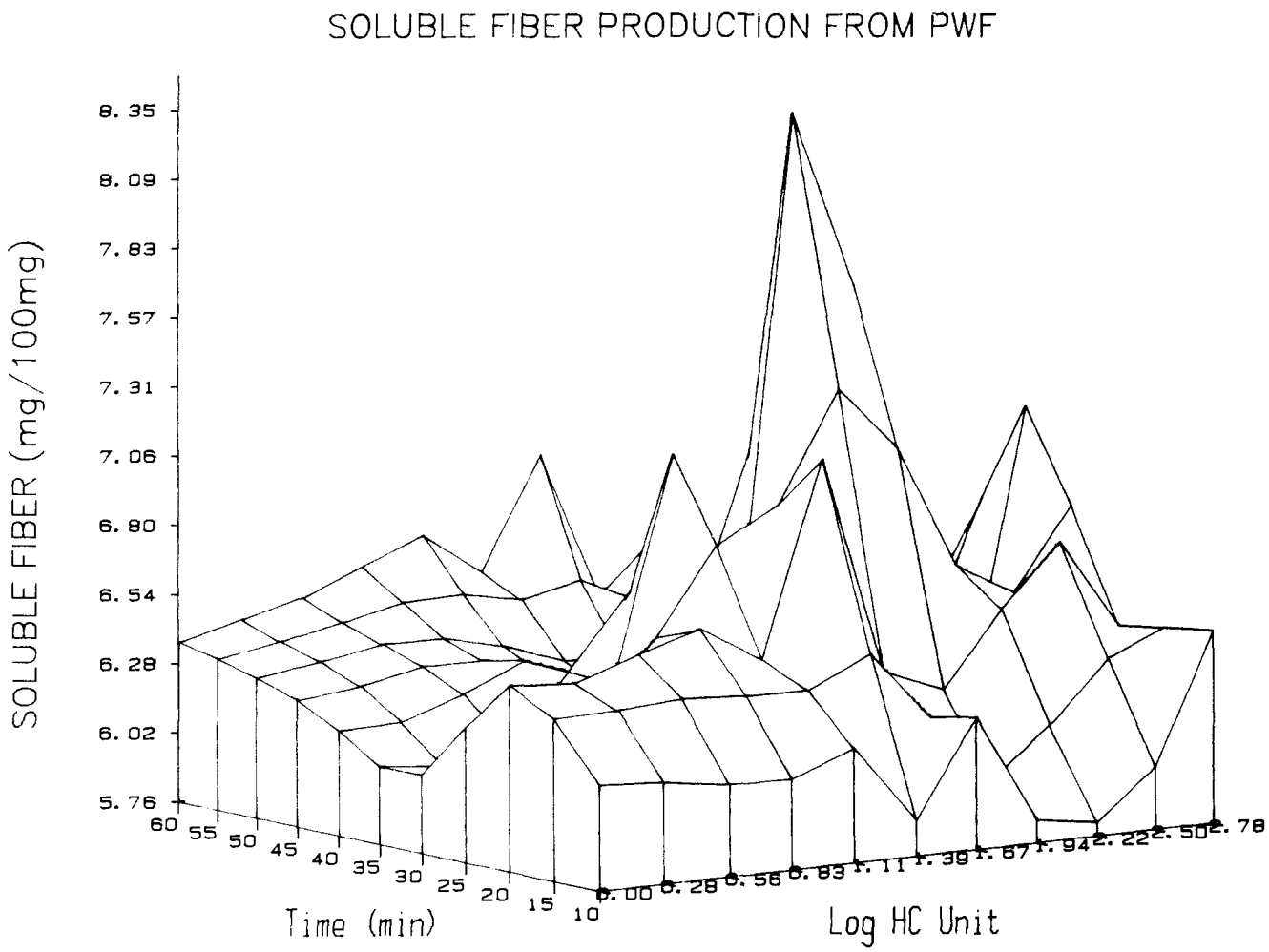

Figure 3. Modification of purified wheat fiber with hemicellulase. Actual data points represent the mean of four to five values. The 3-dimensional plot was generated by the G3GRID procedure to produce a smoothed spline.

Table II. Comparison of the Fiber Content of Wheat Fiber Fractions (% of Total Dietary Fiber)[a]

fiber fraction	insoluble fiber	soluble fiber
crude wheat fiber (CWF)[b]	83.4 ± 5.7	15.8 ± 6.6
purified wheat fiber (PWF)[c]	84.1 ± 3.8	15.9 ± 3.7
modified wheat fiber (MWF)[d]	69.6 ± 7.2	30.1 ± 0.7

[a] Data are expressed as the $X \pm$ SE for three to five individual determinations. [b] The percent insoluble and soluble fiber in the CWF is based on the actual fiber content of CWF and does not include the contribution of protein, fat, and carbohydrate to the total weight. [c] PWF is defined as the fraction remaining after the removal of protein, fat, and carbohydrate. [d] PWF modified in the presence of 60 HCU/mL per g of PIFF hemicellulase.

are summarized in Table II. The tabulated figures for insoluble and soluble fiber are mean values of three to five determinations. The soluble fiber content of the CWF, as a percent of total fiber, was 16% and remained constant during the purification process (PWF). A further increase in the soluble fiber content was achieved by modification of the PWF fraction. Modification of the PWF fraction under the conditions described above resulted in an increase in the soluble fiber fraction from 16% in the CWF or PWF fractions to 30% in the MWF fraction (87% increase). Loss of fiber as a result of hydrolysis was less than 4.07 ± 0.09%. Caprez et al. (1987) have reported a 70% and 19% increase in soluble fiber in yellow pea hulls and apple pomace, respectively, using a combination of Pectinex/Celluclast. However, a 5–25% reduction in total dietary fiber was also reported. These differences could be attributed to a difference in enzyme preparation or incubation conditions.

The present study demonstrated that the soluble fiber content of wheat fiber could be increased by enzymatic hydrolysis of the purified insoluble fiber component. The process has been used for production of a modified wheat fiber, which has been used in a feeding trial examining the physiological properties of the modified fibers.

ACKNOWLEDGMENT

We thank J. A. Maga and C. H. Kim for their technical advice and assistance.

LITERATURE CITED

Blackburn, N. A.; Redfern, J. S.; Jarjis, H.; Holgate, A. M.; Hanning, I.; Scarpello, J. H. B.; Johnson, I. T.; Read, N. W. The mechanism of action of guar gum in improving glucose tolerance in man. *Clin. Sci.* **1984**, *66*, 329–333.

Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–256.

Caprez, A.; Arrigoni, E.; Neukom, H.; Amado, R. Improvement of the Sensory Properties of Two Different Dietary Fibre Sources through Enzymatic Modification. *Lebensm.-Wiss.-Technol.* **1987**, *20*, 245–249.

Demigne, C.; Remesy, C. Stimulation of Absorption of Volatile Fatty Acids and Minerals in the Cecum of Rats Adopted to a Very High Fiber Diet. *J. Nutr.* **1985**, *115*, 53–60.

Folch, J.; Lees, M.; Sloan-Stanley, G. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 466.

Grundleger, M. L.; Oh, Y. N. The Effect of Enzymatic Modification of Wheat Bran on the Digestibility of Fiber and the Production of Short Chain Fatty Acids. Submitted for publication in *J. Nutr.* **1989**.

Jenkins, D. J. A.; Goff, D. V.; Leeds, A. R.; Alberti, K. G. M. M.; Wolever, T. M. S.; Gassul, M. A.; Hockaday, T. D. R. Unabsorbable Carbohydrates and Diabetes: Decreased Postprandial Hyperglycemia. *Lancet* **1976**, *24*, 172–174.

Jenkins, D. J. A.; Leeds, A. R.; Gassul, M. A.; Cochet, B.; Alberti, K. G. M. M. Decrease in Postprandial Insulin and Glucose Concentrations by Guar and Pectin. *Ann. Intern. Med.* **1977a**, *86*, 20–23.

Jenkins, D. J. A.; Wolever, T. M. S.; Hockaday, T. D. R.; Leeds, A. R.; Howarth, R.; Bacon, S.; Apling, E. C.; Dilawari, J. Treatment of Diabetes with Guar Gum. *Lancet* **1977b**, *2*, 779–780.

Jenkins, D. J. A.; Wolever, R.; Taylor, R. H.; Bacon, S.; Hockaday, T. D. R. Dietary fiber and ketone bodies: Reduced urinary 3-OH excretion in diabetics on guar. *Br. Med. J.* **1979**, *2*, 1555–1561.

Jenkins, D. J. A.; Wolever, T. M. S.; Taylor, R. H.; Barker, H. M.; Fielden, H.; Jenkins, A. L. Effect of guar crispbread with cereal products and leguminous seeds on blood glucose concentrations of diabetics. *Br. Med. J.* **1980**, *2*, 1248–1250.

Monte, W. C.; Maga, J. A. Extraction and Isolation of Soluble and Insoluble Fiber Fractions from the Pinto Bean (*Phaseolus vulgaris*). *J. Agric. Food Chem.* **1980**, *28*, 1169–1174.

Prosky, L.; Asp, N.-G.; Furda, I.; Devries, J. W.; Schweizer, T. F.; Harland, B. F. Vitamins and Other Nutrients: Determination of Total Dietary Fiber in Foods, Food Products and Total Diets: Interlaboratory Study. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 1044–105.

SAS/System v 6.03, SAS Institute Inc., Cary, NC, 1988.

Simpson, R. W.; Mann, J. I.; Eaton, J.; Carter, R. D.; Hockaday, T. D. R. High-carbohydrate diets and insulin-dependent diabetics. *Br. Med. J.* **1979a**, *2*, 523–525.

Simpson, R. W.; Mann, J. I.; Eaton, J.; Moore, R. A.; Carter, R.; Hockaday, T. D. R. Improved glucose control in maturity-onset diabetes treated with high-carbohydrates-modified fat diet. *Br. Med. J.* **1979b**, *1*, 1753–1756.

Southgate, D. A. T. Use of the Southgate Method for Unavailable Carbohydrates in the Measurement of Dietary Fiber. In *Basic and Clinical Nutrition: The Analysis of Dietary Fiber in Food*; James, W. P. T., Theander, O., Eds.; Marcel Dekker: New York, 1981; Vol. 3.

Tulung, B.; Remesy, C.; Demigne, C. Specific Effects of Guar Gum or Gum Arabic on Adaptation of Cecal Digestion to High Fiber Diets in the Rat. *J. Nutr.* **1987**, *117*, 1556–1561.

Van Soest, P. J.; Robertson, J. B.; Roe, D. A.; Riveres, J.; Lewis, B. A.; Hackler, L. R. The Role of Dietary Fiber in Human Nutrition. *Proc. Cornell Nutr. Conf.* **1978**, 5–12.

Received for review February 22, 1989. Revised manuscript received December 22, 1989. Accepted January 1, 1990.

Registry No. Gammanase 1.5L, 9025-56-3.

Potentiation of *Bacillus thuringiensis* Insecticidal Activity by Serine Protease Inhibitors

Susan C. MacIntosh,* Ganesh M. Kishore, Frederick J. Perlak, Pamela G. Marrone, Terry B. Stone, Steve R. Sims, and Roy L. Fuchs

Plant Science Technology, Monsanto Agricultural Company, 700 Chesterfield Village Parkway, St. Louis, Missouri 63198

Several serine protease inhibitors enhanced the insecticidal activity of the insect control proteins from *Bacillus thuringiensis* var. kurstaki, var. tenebrionis, and var. israelensis against their target insects, tobacco budworm and other lepidopterans, Colorado potato beetle, and mosquito, respectively. *B. thuringiensis* protein concentrations, at levels causing minimal insect mortality (10–20%), were mixed with purified protease inhibitors and assayed against the appropriate target insects. The presence of extremely low levels (4 μM) of protease inhibitors enhanced the insecticidal activity of *B. thuringiensis* var. kurstaki by 2–20-fold. Protease inhibitors also potentiated the insect growth inhibitory activity of sublethal *B. thuringiensis* var. kurstaki protein concentrations. Genetically improved tobacco plants expressing a protease inhibitor fused to a truncated *B. thuringiensis* insect control protein showed levels of activity enhancement similar to those seen with purified protease inhibitors.

A variety of *Bacillus thuringiensis* (Bt) strains have been isolated, which produce proteins active against a wide range of insects including lepidopterans, coleopterans, and dipterans (Klausner, 1984). Bt is a Gram-positive, spore-forming bacterium that characteristically produces a parasporal crystal protein that accounts for this insecticidal activity. Crystal and spore preparations of the lepidopteran-active *B. thuringiensis* var. kurstaki (Btk) have been used as commercial insecticides for many years. A second class of Bt proteins, produced by strains exemplified by *B. thuringiensis* var. israelensis (Bti), are active against dipteran insects (Thomas and Eller, 1983; Tyrell et al., 1979). Recently, a new class of Bt proteins, active against coleopteran insects, was isolated from *B. thuringiensis* var. tenebrionis (Btt) (Krieg et al., 1983) and *B. thuringiensis* var. san diego (Btsd) (Herrnstadt et al., 1986). Classification of these three crystal protein types Btk, Btt, and Bti are identified as cryI, cryIII, and cryIV, respectively (Höfte and Whiteley, 1989).

All three classes of Bt proteins have extremely short

0021-8561/90/1438-1145$02.50/0