

DBT Study Title: Pepsin Digestibility Assay

Dow AgroSciences LLC Study Titles: *In Vitro* Simulated Gastric Fluid Digestibility Study of Microbially Derived Cry1F(synpro), Unpublished Report of Dow AgroSciences LLC (Study ID 01008). *In Vitro* Simulated Gastric Fluid Digestibility Study of Microbially Derived Cry1A (synpro), Unpublished Report of Dow AgroSciences LLC (Study ID 010026).

Introduction

These studies were conducted to evaluate the *in vitro* digestibility of Cry1F protein and Cry1Ac protein in simulated gastric fluid (SGF). Bovine serum albumin was used as a positive control since it is known to degrade readily in SGF, and beta-lactoglobulin was used as a negative control since it is known to persist in SGF. Proteins were subjected to SGF for specified time intervals and then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Additionally, Western blot analysis was also performed for Cry1F and Cry1Ac proteins.

Quality Assurance. The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit of Dow AgroSciences LLC in Indianapolis, Indiana.

Archiving. Raw data and the original copy of the final report are archived at Dow AgroSciences' testing facility archives in Indianapolis, Indiana.

Materials and Methods

Test Substances. Separate lyophilized powders containing 15% Cry1F or 14% Cry1Ac, isolated from recombinant *Pseudomonas fluorescens*, were used in these studies. BSA and beta-lactoglobulin were obtained from Sigma Chemical ($\geq 96\%$ purity and $\geq 90\%$ purity, respectively). Simulated gastric fluid (SGF) containing approximately 0.3% (w/v) pepsin was prepared and adjusted to a pH of approximately 1.2 as described in the United States Pharmacopeia.

Protein Digestibility. The digestibility of Cry1F, Cry1Ac, BSA and beta-lactoglobulin was determined by adding aliquots of aqueous formulations of the individual proteins to SGF. Digestions were performed at time intervals of 1, 3, 6, 10, 15, 20, 30 and 60 minutes in a water bath set to 37 °C. After each specified incubation interval, an aliquot of the reaction mixture was removed and added to a stop solution of sodium carbonate. The stopped reactions were then placed on ice until all of the time points were sampled for the proteins.

Analysis of Protein Digestion. SDS-PAGE and Western blot analysis was used to assess the extent of Cry1F and Cry1Ac digestion. BSA and beta-lactoglobulin digestion was assessed by SDS-PAGE.

Results

Cry1F Protein. Digestion results for Cry1F are shown in Table 1. Cry1F was digested in less than one minute, as demonstrated by both SDS-PAGE and Western blot analysis. The positive and

negative controls (BSA and beta-lactoglobulin) responded as expected. BSA was digested before the one minute time point while beta-lactoglobulin remained undigested for 60 minutes (the duration of the experiment).

Table 1. *In Vitro* Digestibility of Cry1F in SGF

Protein	Digestion Demonstrated By	
	SDS-PAGE	Western Blot Analysis
BSA	< 1 minute	N/A ¹
Beta-lactoglobulin	>60 minutes	N/A ¹
Cry1F	< 1 minute	< 1 minute

¹N/A = Not analyzed.

Cry1Ac Protein. Digestion results for Cry1Ac are shown in Table 2. Cry1Ac was digested in less than one minute, as demonstrated by both SDS-PAGE and Western blot analysis. The positive and negative controls (BSA and beta-lactoglobulin) responded as expected. BSA was digested before the one minute time point while beta-lactoglobulin remained undigested for 60 minutes (the duration of the experiment).

Table 2. *In Vitro* Digestibility of Cry1F in SGF

Protein	Digestion Demonstrated By	
	SDS-PAGE	Western Blot Analysis
BSA	< 1 minute	N/A ¹
Beta-lactoglobulin	>60 minutes	N/A ¹
Cry1Ac	< 1 minute	< 1 minute

¹N/A = Not analyzed.

Conclusions

These results confirm that Cry1F and Cry1Ac proteins are readily digested by pepsin (<1 minute) under simulated gastric conditions (pH 1.2) as demonstrated by both SDS-PAGE and Western blot analysis.