

Title: Validation of Agent Inactivation and Isolation from Food Systems**Principal Investigator:** Alvin Lee, Illinois Institute of Technology**Center of Excellence:** Food Protection and Defense Institute (FPDI)**COE Lead/Co-Lead Institution:** University of Minnesota**Project Start Date:** 09/2011**Project Completion Date:** 06/2014**Project Status:** Complete**Research Theme:** Agent Behavior**Participating State(s):** Illinois, Minnesota**Amount Awarded to Date:** \$357,321**Award Number:** 2010-ST-061-FD0001

Abstract: Validation of a process needed to eliminate a microbiological or chemical hazard in food can be difficult. Processing technologies that have shown efficacy in eliminating a hazard under laboratory conditions, usually under well controlled parameters, may not be effective against the threat agent during scale-up. Often there are no proven procedures that can be immediately applied to novel processing technologies, which means that validation procedures must be developed and tested. This project involves integration of projects, current and previous, funded by NCFPD so that validation of developed protocols by the various research groups, that were laboratory based, could be brought together and conducted in a secure high-level biocontainment facility at IFSH that houses pilot or full scale food processing equipment. The project team worked with pilot-scale HTST processing equipment to validate thermal inactivation of ricin and anthrax in large scale based on previously developed laboratory-scale protocols. In addition, sanitation and disinfection protocols were validated giving the food industry data and conditions to inactivate ricin and anthrax and sanitize processing equipment prior to processing food for human consumption. The results showed typical pasteurization conditions for fluid milk are insufficient to inactivate ricin and anthrax if present in nonfat milk and complete inactivation of ricin and anthrax would require considerably longer hold times that would result in loss of milk quality. However, UHT conditions used to process fluid milk could inactivate anthrax and ricin provided if specific processing conditions were achieved and maintained. These results suggest a significant vulnerability for pasteurized fluid milk supply to a contamination event with both biothreat agents.

Project Type: Research**End User Engagement:**

- Academic Community
- DHS Labs
- DHS Science and Technology Directorate
- DHS U.S. Customs and Border Protection
- Federal Bureau of Investigation

Executive Summary (2014): Validation of a process needed to eliminate a microbiological or chemical hazard in food can be difficult. Studies that have shown that scale-up of a process may not necessarily correspond to the same reduction of the hazard as those determined in well-controlled, laboratory studies. This project involves integration of projects aimed at evaluating the thermal and chemical stability of ricin and *Bacillus anthracis* in a secure high-level biocontainment facility at IFSH that houses pilot- or full-scale food processing equipment for scale-up and validation activities. The first validation study evaluated the effects of thermal processing on the stability of crude and purified ricin using a pilot-scale HTST unit. The processing conditions (times and temperatures) that were examined in this study

were similar to those used in a previous study which determined the half-life values of ricin in the milk-based infant formula. In this early study, loss of toxicity and ELISA detection of ricin was determined after the toxin was heated in sealed, immersed vials in a well-controlled environment (water bath). In the present validation study, ricin (crude and purified) stability in reconstituted nonfat milk was evaluated using the HTST configured to process milk under conditions similar to those used in the previous study. The apparent half-life values for purified ricin processed at four temperatures (80-95°C), as calculated for entire processing treatment, were less than 30 s. These values are at least an order of magnitude lower than those previously reported for ricin inactivation in milk-based infant formula. The differences in half-life values could be due to the compositional differences between the two food matrices, as nonfat milk has lower fat and mineral content than infant formula. In previous studies, the temperature of the product was monitored through the entire process, and the calculations of the kinetic parameters (k and $t_{1/2}$) took into account the come-up time and cool-down times. However, in the current study, it was not possible to correct the kinetic parameters for the come-up time and cool-down times of the HTST. Furthermore, the temperature at the beginning of the hold tube was, on average, 7°C higher than the desired processing temperature, or the temperature at the end of the hold tube. The additional thermal input as a result of the higher initial hold tube temperature is likely partially responsible for the greater inactivation of ricin than that expected from the set processing temperature. Laboratory-derived kinetic parameters for ricin inactivation in addition to estimated temperature profile for product in the HTST were used to predict the amount of ricin inactivated in the HTST under defined processing conditions. An estimate was made of the log reduction of ricin from the temperature profile (estimated) of the product processed at 85°C in the pilot-scale HTST unit at a flow rate where product flow profile was turbulent (2.0 L/min), and using z-values for ricin inactivation in infant formula (Jackson et al., 2006) and in milk (Wang, 2010). The experimentally measured log reduction of ricin using HTST was approximately 1.9 - 3.5 times greater than the estimated value. The greater experimental inactivation may be due to differences in food matrices used in the study and the assumption of linear increases and decreases in product temperature in the heating and cooling sections of the HTST, respectively. The ability to measure the temperature throughout all portions of the HTST would have allowed a more accurate estimation of the log reduction of ricin in milk during processing. The half-life values at processing temperatures of 85 and 95°C for ricin in crude extracts were not substantially different from those of the highly purified toxin. The slight differences in the thermal stability of ricin in crude (laboratory-prepared) vs. purified (purchased from Vector Laboratories) extracts may be due to differences in ricin isoforms in the extracts, and the cultivars of *Ricinus communis* used to prepare the extracts. Since the different forms of ricin have slightly different amino acid sequences, it is expected that their thermal stabilities may differ slightly as do their toxicities. Cytotoxicity analyses are being conducted at FDA/National Center for Toxicological Research (NCTR) on the thermally processed milk samples containing ricin. These analyses, which will be completed by the end of March 2015, will provide a more accurate measurement of the biological activity of ricin in the processed milk samples. However, as shown in studies by Jackson et al. (2006, 2010), the thermal stability of ricin, as measured by ELISA detection is not significantly different from the stability of the toxin as measured with a cell toxicity assay. A final report on the cytotoxicity results will be submitted in spring 2015. *Bacillus anthracis* spores were inoculated into large quantities of reconstituted skim milk powder and processed by a MicroThermics HTST Bantam 1S. The spores were processed at 105, 110 and 115 °C and at various hold temperatures to determine if previous laboratory based data on thermal inactivation could be scaled-up and determine if real world processing parameters could inactivate *B. anthracis* spores. The targeted processing temperatures at the end of the hold tube within the HTST will require the HTST to ramp up heating above the targeted temperature by approximately 7 °C at the start of the hold tube. This heating profile within the HTST resulted in the *B. anthracis* spores being exposed to different temperatures within the hold tube and the inactivation rates were not uniformly distributed throughout the hold tube. Therefore, the D-values and time to achieve a 6-log inactivation will be in the range of the D-values at the targeted temperature to the D-values of the targeted temperature plus 7 °C. At the processing temperature of 105 °C, a 15 s hold time was required to achieve a 6-log inactivation and

temperatures within the hold tube ranged from 112 °C at the start of the hold tube to 105 °C at the end of the hold tube. At the processing temperature of 110 and 115 °C, a hold time of 5 and 2 s were required to achieve a 6-log inactivation of *B. anthracis* spores. In addition to the processing temperature and hold times, the Re values of >8,000 and turbulent flow type were other important parameters that contribute to inactivation. The data from the project suggests that the processing and heating profiles of the HTST, when operated under specific conditions could achieve a 6-log inactivation of *B. anthracis* spores in skim milk. The data can be translated to the food industry for use because the HTST unit used by the project team is one of the models currently in use by food companies. The outcome of the project is also unique as this is probably the first report of using *B. anthracis* spores in large quantities of skim milk and processed under biocontainment conditions using pilot scale HTST processing equipment. Overall, the results of the thermal inactivation study reported in this study indicated that although milk pasteurized with Microthermics HTST unit was over-processed, the conditions typically used to pasteurize fluid milk (72°C for 15 s; 89°C for 1.0 s; 90°C for 0.5 s) would not be sufficient to ensure complete inactivation of ricin and anthrax if they were to be present in nonfat milk. To ensure inactivation of ricin, considerably longer hold times in the HTST would be required, which likely result in reduction of milk quality. These hold times would be several minutes at 80 and 85°C, ~30 s at 90°C and ~21 s at 95°C. However, at UHT conditions used to process fluid milk, typically 135-138 °C for up to 3 s, anthrax and possibly ricin could be inactivated provided the minimal flow rates to ensure turbulent flow at specific Re value were achieved and maintained. These results suggest a significant vulnerability for pasteurized fluid milk supply to a contamination event with anthrax and ricin.

Peer-reviewed journal articles produced from this project

Citation

Jackson, Lauren S., Odbert A. Triplett, and William H. Tolleson. "Influence of Yogurt Fermentation and Refrigerated Storage on the Stability of Protein Toxin Contaminants." *Food and Chemical Toxicology* 80 (June 2015): 101-107. doi:10.1016/j.fct.2015.03.003.

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