

MAFMA Final Report

Project Title Screening for a *Clostridium botulinum* surrogate spore for validation of low-acid high-pressure processes

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Please complete all questions below and attached form

1. **Objective Summary** (1-2 sentence summary) Evaluate pressure-thermal resistance of selected non-pathogenic *Bacillus* and *Clostridium* spores and estimate their microbial kinetic inactivation parameters.

2. Objective Accomplishments

(If objectives were not met, what extenuating circumstances contributed to that factor?)

Convey all of your progress on this project including that obtained with the industry and other matching funds.

- Custom fabricated a mini high-pressure microbial kinetic tester through Avure Technologies, Kent, WA. The unit was rated to 700 MPa pressure and 130°C process temperature. It has a 54 ml stainless steel pressure chamber immersed in a temperature-controlled bath, and the system is pressurized by an intensifier. The bath surrounding the pressure chamber was maintained at a suitable temperature so that isothermal-process conditions could be maintained throughout the pressure-holding time.
- Thermal inactivation of the spores were determined using custom-fabricated aluminum tubes (12 mm i.d., 42 mm height, 3 mm wall thickness) through OSU machine shop.
- Our study focused on characterizing combined pressure-temperature resistance of spoilage spores. A parallel study is being conducted by National Food Laboratories, Dublin, CA on inactivation of *Clostridium botulinum* spores under the sponsorship of Dual Use Science and Technology program sponsored by US Army labs and food companies. DUST consortium and NFL agreed to share non-confidential information with OSU investigators related to methods and protocols for conducting spore inactivation studies. This will facilitate meaningful comparison of kinetics of surrogate destruction against pathogenic spores, and provide an expanded base of reliable and credible scientific data in this area.
- Evaluated pressure-thermal resistance of wide variety of *Bacillus* and *Clostridia* spores. Strains of *Clostridium tyrobutylicum* (ATCC 25755), *Thermoanaerobacterium*

thermosaccharolyticum (ATCC 27384), and *Clostridium sporogenes* (ATCC 7955), *Bacillus polymyxa*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* (ATCC 49763, ATCC 23350, and ATCC 53495) were either purchased from the American Type Culture Collection (Manassas, VA, USA) or obtained from Ohio State University culture collections. Strains of *Bacillus amyloliquefaciens* TMW 2.479 Fad 82 and *Bacillus amyloliquefaciens* TMW 2.482 Fad 11/2 originally isolated from ropy bread (Röcken and Spicher, 1993) and provided by Dr. Michael Gänzle, Lehrstuhl für Technische Mikrobiologie, Technische Universität München (Freising, Germany). Strain of *Bacillus sphaericus* NZ 14 was provided by Dr. Rosalind Robertson, Fonterra Research Centre (Palmerston North, New Zealand). The food substrate effect was studied using deionized water, egg patties, milk, beef and mashed potato. In addition, PATP inactivation of natural spores present in red pepper, black pepper, and garlic powder was also investigated.

Key Results

The spores of *Bacillus* spp. and *Clostridium* spp. used in this study exhibited different inactivation patterns during thermal and PATP treatment. PATP-treated spores clearly showed divergence from linear kinetics and exhibited a nonlinear behavior with rapid initial inactivation immediately after pressure-come-up time, followed by a characteristic tailing during extended pressure-holding times. This may indicate that PATP has multiple targets of action in bacterial spore.

Effect of process come-up time on inactivation of bacterial spores

Three anaerobic *Clostridium* spp. and six aerobic *Bacillus* spp. tested varied in their resistance to thermal and PATP treatment during the process come-up time. For most of *Clostridium* and *Bacillus* spores tested, no significant come-up time log reduction were observed during thermal treatment at 105°C. However, when the temperature increased to 121°C, both *Clostridium* and *Bacillus* spores had significant log-reductions ($p < 0.05$) during the come-time. Increase in process pressure from 0.1 MPa to 700 MPa at constant temperature also increased microbial lethality of both *Clostridium* and *Bacillus* spores during come-up time.

Bacterial spore resistance for thermal and pressure-assisted thermal processing

During PATP treatment, pressure accelerated considerably the inactivation of various bacterial spores compared to thermal treatment alone. The reduction of laboratory grown or natural spores isolated from foods increased with increasing pressure and temperature.

The anaerobic and aerobic spore-forming bacteria were reduced during the pressure come-up time. PATP treatment (700 MPa and 121°C for 1 min) was sufficient to completely inactivate up to 7-8 log reduction for all the spores tested. Among the spores evaluated, *T.*

thermosaccharolyticum, *B. amyloliquefaciens* Fad 82, and *B. amyloliquefaciens* Fad 11/2 were identified as the most PATP resistant organisms.

Inactivation kinetic parameters of bacterial spores

Inactivation kinetic parameter (D), inactivation parameters from the non-linear, Weibull model were estimated various spores were estimated. In addition, temperature coefficient (z_T), and pressure coefficient (z_p), of *B. amyloliquefaciens* Fad 82 one of the most resistant surrogate spore were also estimated.

As expected, thermal treatment at 105°C showed the highest D and lowest b values. In general, D values decreased with increasing temperature or pressure, while b values increased with increasing temperature or pressure. PATP caused the varied inactivation curves as compared to thermal treatment, resulting in a broad range of shape factors. Similarly, the inactivation patterns spores in different food matrices followed nonlinear behavior, including a slope tailing as indicated by the shape factors. D values were inversely related to Weibull model parameter b values. At 105°C, increasing from pressure from 0.1 MPa to 700 MPa accelerated the inactivation of *Clostridium* and *Bacillus* spores. Similarly, the inactivation of *Clostridium* and *Bacillus* spores was increased with increasing the level of pressure at 121°C. However, the magnitude of pressure lethality reduced at elevated process temperature of 121°C. This is possibly due to the increased impact of 121°C thermal treatment alone on the inactivation of *Clostridium* and *Bacillus* spores. z_p value of *B. amyloliquefaciens* spores increased from 170 MPa at 95°C to 332 MPa at 121°C, suggesting that spores became less responsive to pressure changes at higher temperatures. Similarly, z_T value increased from 8.2°C at 0.1 MPa to 26.8°C at 700 MPa, indicating that at elevated pressures, the spores were less responsive to changes in temperature.

3. **Unexpected findings** if any none

4. **Practical impacts of research efforts.** Include: implementation of accomplishments by industry partners (if any), identification of economic impacts, and any further pursuit by PI of research in area of this project whether MAFMA or not.

- a. **Short Term Impacts** MAFMA funds helped principal investigator to establish a research program investigating pressure-assisted thermal processing of foods with emphasis on spore inactivation and process uniformity studies. Pressure-thermal resistance of range of bacterial spores have been evaluated under controlled pressure-thermal conditions. Research findings so far suggest that pressure does not appear to offer any protective effect on the spores tested. Combined pressure-temperature appear to accelerate the inactivation of bacterial spores.
- b. **Long Term Impacts:** Identification of a surrogate organism can help validate pressure assisted thermal processing for many low-acid shelf stable foods that are traditionally thermally processed. Since pressure does not affect smaller molecules, pressure treated foods will have better flavor and vitamin content than thermally processed foods. Further, the technology can also be applied to create new products where it is critical to process at lower temperatures to avoid damage to texture.

5. If you are also making reports to other funding agencies in the course of this research work, please include a copy of that report.

Attached.

6. If any publications resulted from the research, a copy must be included. Please note we were notified by the USDA/CSREES National Program Leader for the Midwest Advance Food Manufacturing Alliance (MAFMA) that all publications resulting from research that was funded by MAFMA must include the following wording **“The project was supported by the USDA Cooperative State Research, Education and Extension Service, special research grant number 200X-34328-xxxxx.**

Copy of the manuscript submitted to Journal of Food Protection (in review) is attached.

Related Publications from the lab

Rajan, S., J. Ahn, V. M. Balasubramaniam and A. E. Yousef. 2005. Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores in mashed egg patties. Journal of Food Protection (submitted).

Ahn, J. V.M. Balasubramaniam, and A.E. Yousef . 2005. Inactivation kinetics of selected heat resistant aerobic and anaerobic bacterial surrogate spores by pressure-assisted thermal processing. International Journal of Food Microbiology (in preparation).

Ahn, J. and V.M. Balasubramaniam. 2005. Inactivation of natural spores by pressure assisted thermal processing (In preparation).

Presentations

Ahn, J. and V.M. Balasubramaniam. 2005. Inactivation of natural spores by pressure assisted thermal processing. Abstract no. 17D-21. Annual Meeting of Institute of Food Technologists, New Orleans, LA. July 16-20.

Ahn, J., V.M. Balasubramaniam, and A.E. Yousef. 2005. Effect of pressure-assisted thermal processing on the inactivation of selected *Clostridium and Bacillus* surrogate spores. Poster presentation abstract. Nonthermal Processing Workshop, Co-sponsored by Institute of Food Technologists, Nonthermal Processing Division and EFFoST. USDA Eastern Regional Research Center, Philadelphia, PA. September 15-16.

Balasubramaniam, V.M. 2005. High pressure processing. Short course on Advanced Process Technologies, University of California, Davis, CA. April 4-5.

Balasubramaniam, V.M. 2005. Food preservation by high pressure processing: Opportunities and challenges. Institute of Food Technologists, Ohio valley section suppliers expo lecture series. Sharonville, OH. April 14.

Balasubramaniam, V.M. 2005. High hydrostatic pressure processing. Current status and research needs. Presented at "Emerging Food Processing Technologies Workshop, From the Lab Bench to the Table," U.S. Department of Agriculture Cooperative State, Research, Education, and Extension Service (USDA CSREES), Washington, DC. May 26-27.

Srilatha Pandrangi, Sandeep Rajan, V.M. Balasubramaniam and A.E. Yousef. 2005. Combined Pressure-Thermal Resistance of selected *Bacillus* spores. Abstract no. 54F-14, Annual Meeting of Institute of Food Technologists, New Orleans, LA. July 16-20.

Rajan, S., V. M. Balasubramaniam, S. Pandrangi, and A. E. Yousef. 2005. Inactivation of *Bacillus stearothermophilus* spores in egg patties by pressure-assisted thermal processing. Abstract no. 53F-11. Annual Meeting of Institute of Food Technologists, New Orleans, LA. July 16-20.

Rajan, S., J. Ahn, V. M. Balasubramaniam, and A. E. Yousef. 2005. Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores. Poster presentation abstract. Nonthermal Processing Workshop, Co-sponsored by Institute of Food Technologists, Nonthermal Processing Division and EFFoST. USDA Eastern Regional Research Center, Philadelphia, PA. September 15-16.

Rajan, S., J. Ahn, V.M. Balasubramaniam and A.E. Yousef. 2005. Modeling pressure and thermal sensitivity of *Bacillus amyloliquefaciens* spores in mashed egg patties during pressure-assisted thermal processing. Abstract no. 572b. Topical conference on food engineering, 2005 Annual Meeting of Chemical Engineers, Cincinnati, OH. October 30-November 4.

Theses and Miscellaneous Reports

Sandeep Rajan. 2005. Inactivation kinetics of bacterial spores in egg patties by pressure-assisted thermal processing. MS Thesis. Food Science and Nutrition. Ohio State University.