Modeling Pressure and Thermal Sensitivity of Bacillus Amyloliquefaciens Spores in Mashed Egg Patties during Pressure-Assisted Thermal Processing

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ABSTRACT:
Bacillus amyloliquefaciens is a potential surrogate for *Clostridium botulium* in validation studies involving spore inactivation by pressure-assisted thermal processing (PATP). Spores of *B. amyloliquefaciens* Fad 82 were inoculated into mashed egg patties (10^8 spore/g), and the product was treated by combinations of pressure (0.1 MPa-700 MPa) and heat (95°C-121°C) in a custom-made high-pressure kinetic tester. Inactivation kinetic parameter (D), temperature coefficient (z_T), and pressure coefficient (z_p) values were determined using a linear model. The increase in process pressure decreased the D value at 95°C, 105°C and 110°C considerably; however, at 121°C, the contribution of pressure to spore lethality was less pronounced. z_p value increased from 170 MPa at 95°C to 332 MPa at 121°C, suggesting that *B. amyloliquefaciens* 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. The test product (egg patties) is a suitable food matrix for PATP application in food processing.

Keywords: Pressure-assisted thermal processing; *Bacillus amyloliquefaciens*; egg; pressure and temperature coefficients; kinetic models.

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INTRODUCTION

Thermal sterilization of egg products has severe limitations. Such a treatment results in the development of thermally-induced off-flavors, syneresis, and a green-gray discoloration of the egg products due the formation of iron-sulfur compounds *(3)*. When conventional thermal retorting and PATP treatment were compared, the latter produced a more acceptable egg product due to a shorter exposure time to heat *(2)*. Establishing a safe and efficient PATP sterilization process requires a proper definition of inactivation kinetic parameters for various target pathogenic and spoilage microbes, under pressure, heat, and their combinations. Limited studies addressed PATP spore inactivation kinetics over a range of pressure and temperature conditions (*6, 9*). Hence, the pressure and temperature coefficients for inactivation of bacterial spores during PATP are yet to be characterized. This knowledge would be extremely useful in developing a safe PATP process for a specific application. Since *B. amyloliquefaciens* Fad 82 produces PATP-resistant spores, researchers proposed using this bacterium as a surrogate for *Clostridium botulinum* spores. Therefore, spores of this bacterium were chosen in this investigation.

MATERIALS AND METHODS

Spore suspensions of *B. amyloliquefaciens* Fad 82 (10^9 spores/ml**)** were prepared as described by Margosch et al. *(5).* Egg patties obtained from a commercial egg producer were inoculated with 0.1 ml of spore suspension to get a final spore concentration of ~10^8 spores/g.

Thermal inactivation of spores

The thermal inactivation of *B. amyloliquefaciens* spores in egg patties was determined at 95^{o}C, 105^{o}C, 110^{o}C and 121^{o}C, using custom-fabricated aluminum tubes (*4*). A sample of a mashed egg patties (0.9 g) was transferred into each aluminum tube and 0.1 ml (~10^9 spores/ml) of the spore suspension was added to get a final spore concentration of approximately 10^8 spores/g egg patty. Six tubes were then submerged simultaneously into a 28-liter circulating oil bath (Fisher Scientific), which was maintained at the desired target temperature. The sample temperature was monitored and recorded using a K-type thermocouple (Omega Engineering, Stamford, CT, U.S.A.) attached to a data logger (IOtech, Cleveland, OH, U.S.A.). The heating time (come-up time) was recorded when the target temperature of the sample was reached. The process come-up times were approximately 3.58 min for 95^{o}C, 3.33 min for 105^{o}C, 3.25 min for 110 ^{o}C and 3.30 min for 121^{o}C. The first aluminum tube was removed from the oil bath at the end of come-up time. The other aluminum tubes were subjected to five different hold times; the hold time intervals being different for each target temperature. After the thermal treatments, the sample-containing tubes were immersed promptly into an ice-water bath to avoid further inactivation. Spores surviving the thermal treatment were enumerated as described later.

Pressure-assisted thermal processing of spores

High barrier pouches (5 x 2.5 cm each) made from sterile filter bags (01-002-57, Fisher Scientific) were used as sample holders. A sample of mashed egg patties (0.9 g) and 0.1 ml of the spore suspension (~10^9 spores/ml) were placed inside these high barrier pouches to get a final spore concentration of approximately 10^8 spores/g egg patty. The pouches were then heat-sealed (Impulse Food Sealer, American International Electric, Whittier, CA, U.S.A) and the contents of the pouch were mixed thoroughly. The packaged samples were then placed in a sample carrier consisting of a 10-ml capacity polypropylene syringe (Model 309604; Becton, Dickinson and Company) covered with two layers of insulating material. Water was used as the pressure transmitting fluid within the syringe. Prior to pressurization experiments, the sample carrier containing the spore-inoculated egg patty was preheated in a water bath (Isotemp 928; Fisher Scientific) to a suitable preprocessing temperature. Preheated sample carrier was then immediately loaded into the chamber of the pressure kinetic tester and the pressurization was started when the sample temperature reached a predetermined value. The test samples were subjected to a combination of process temperatures (95^{o}C, 105 ^{o}C, 110 ^{o}C, and 121 ^{o}C) and pressures (500, 600, and 700 MPa) for upto 15 min. The process hold-time intervals were adjusted for each combination of process pressure and temperature so that adequate data points were collected for subsequent data analysis. The process hold-times did not include the pressure come-up or the de-pressurization times. After depressurization, the samples were cooled immediately in an ice-water bath. The untreated control samples (non-pressure treated inoculated egg patties) were heated at 80^{o}C for 30 min, to activate the spores for enumeration purposes. Pouches containing the inoculated egg product (control and pressure-treated) were opened aseptically, and their contents were used for determining the total viable spore count as indicated later.

Enumeration of Survivors

Heat- or PATP-treated sample (1g) was mixed with 9 ml of peptone water (1 g/liter) and homogenized for 2 min in a stomacher (Seward Lab Stomacher, Norfolk, UK). The homogenized sample was further serially-diluted in peptone water and the dilutions (1 ml) were pour-plated using duplicate trypticase soy agar (TSA; BD Difco) plates. The plates were then incubated at 32^{o}C for 48 h before enumeration.

RESULTS AND DISCUSSIONS

In general, the PATP inactivation of *B. amyloliquefaciens* spores exhibited a bi-phasic behavior, with rapid initial inactivation immediately after pressure-come-up time, followed by a characteristic tailing during extended pressure-holding times. The PATP lethality against *B. amyloliquefaciens* spores increased with increase in process pressure at a given temperature or increase in process temperature at a given pressure. The reduction in spore count during pressure-come up time varied between 0.1 and 1.2 log spore/g egg product, depending on the treatment. Lower pressure-thermal combination (e.g., 500 MPa at 95^{o}C-105^{o}C) resulted in no

or limited reduction in the spore count during the pressure come-up time; but application of elevated pressure (> 700 MPa) and temperatures (121^{O}C) inactivated up to 1.2 log spore population during that period. These observations suggests that spores are likely to have different resistances during the pressure-come up time depending on pressure level applied and the time taken to reach target pressure. Resistance of *B. amyloliquefaciens* spores to PATP (D value) was significantly smaller than that to thermal processing, at an equivalent process temperature. However, synergy between heat and pressure diminishes at elevated process temperature, and heat becomes the dominant contributor to the lethality at 121^{O}C. The z_p values of *B. amyloliquefaciens* spores subjected to PATP treatments increased from 170 MPa at 95^{O}C to 332 MPa at 121^{O}C. These results suggest that the spores became increasingly less responsive to pressure changes as the processing temperature increased. Similarly, *B. amyloliquefaciens* spores were more responsive to temperature changes at atmospheric pressure (z_T=8.2^{O}C) than at elevated pressures (z_T = 26.8^{O}C at 700 MPa).

CONCLUSIONS

Different combinations of pressure and temperature bring about similar lethality of *B. amyloliquefaciens* Fad 82 during pressure-assisted thermal processing. The processing resistance parameter, D value, for *B. amyloliquefaciens* spores in egg patties was lower in PATP than in thermal processing at an equivalent temperature. However, *B. amyloliquefaciens* spores became less responsive to pressure changes at 121^{o}C (indicated by the higher Z_p and less negative ΔV value) than at lower temperatures. Furthermore, the responsiveness of *B. amyloliquefaciens* spores to temperature changes, was less at 700 MPa (indicated by the higher z_T and lower E_a value) than at lower pressures.

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