# Inactivation of *Bacillus coagulans* Spores by Pressure-assisted Thermal Processing

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Food sterilization has traditionally been associated with thermal processing at ≥121°C to ensure elimination of pathogenic spore-forming bacteria. Thermal processing alone tends to adversely affect the product's freshness and quality attributes. Pressure-assisted thermal processing (PATP) offers new opportunities to overcome these limitations by treatment of foods at lower temperatures. This study was conducted to examine the lethality of PATP against the spores of *Bacillus coagulans* (an acid-tolerant spoilage bacterium) in a commercial tomato juice. The spores were suspended in deionized water, added to the samples (tomato juice at pH 4.2) at 108 CFU/ml, and treated at 600 MPa and temperatures of up to 105°C for various holding times (≤1.5 min). The viable numbers of spores were subsequently determined on tripticase soy and nutrient agars. A PATP treatment at 100°C for 2 min was found to be sufficient to inactivate the *B. coagulans* spores to an undetectable level (≤10 CFU/ml). The decimal reduction time (D-value) of the spores under these conditions was determined as 30 s. These findings demonstrate the efficacy of PATP treatment against heat and pressure-resistant bacterial spores; however, the level of inactivation will depend on the process conditions and composition of the suspending medium.

## Introduction

Using high pressure to process foods has been known since 1899, although there has not been enough research on the parameters for creating a safe food product for it to have large-scale commercial operations. In pressure-assisted thermal processing (PATP), food products are subjected to pressures ranging from 500 to 700 Megapascals (MPa) at temperatures of 90 to 121ºC for a specific holding time.[10] The food is placed in a flexible package and then vacuumed sealed in order to drive out all of the extra air. The vacuumed sealed package is then placed into a metal tube filled with a liquid such as propylene glycol. Next, using hydraulic fluid, pressure of up to 700 MPa is generated. This pressure is used to drive a piston that reduces the volume in the chamber, therefore increasing the pressure inside of the cylinder. This pressure is then transferred to the product itself, where the product is held under pressure for a few minutes until the microorganisms are rendered inactive.[4] Because of the isostatic pressure, the product is not crushed and maintains its shape and size. Compared to other processing methods, especially thermal processing, there is less thermal damage done to products.[3] High pressure processing retains vitamins and satisfies the public want for fresh foods that use minimal preservatives.

Pressure starts to affect microorganisms between 30 and 50 MPa by influencing gene expression and protein synthesis of the organisms.[9] Acid may be used in combination with high pressure to give further reduction of pathogenic microorganisms. High pressure causes damage to the cells and makes them more suscepti-

**About the Author**

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ble to the effects of acid.[1] Under pressure, dipicolinic acid, the compound responsible for heat resistance, is released from the spore. For example, *Bacillus subtillis* loses 80% of its dipicolinic acid when exposed to pressure of 60 MPa at 30°C. This is one mechanism that facilitates the inactivation of microorganisms under combined pressure and heat.[2]

*Bacillus coagulans* 185A spores were used based on their pressure- and heat-resistance in preliminary experiments. *B. coagulans* spores are frequently isolated from spoiled acidic canned products such as tomato juice and are able to germinate and grow at pH values between 3.7 and 4.5.[6] Like other spore-forming bacteria, such as *Clostridium botulinum*, *B. coagulans* is pressure resistant and relatively heat resistant at acidic pH.[8] Previous studies have shown that B. coagulans has a D-value (the time required to achieve one log cycle of reduction in concentration) of 1.56 min at 100°C in tomato products with a pH of 4.3.[5]

The objectives of this study were to determine the parameters for a pasteurized product using thermal-assisted pressure processing and to calculate the decimal reduction time, D-value, of *B. coagulans* 185A in tomato juice at 100°C.

## Methods

*Bacillus coagulans* 185A culture was obtained from Dr. Dallas Hoover (University of Delaware, Newark, Delaware). *B. coagulans* was grown in trypticase soy broth with 0.6% yeast extract (TSBYE; Difco) with 500 mg/l MnSO4. H2O (Fisher Scientific, Pittsburgh, PA) and 3 mg/l Dextrose (Fisher Scientific, Pittsburgh, PA) at 37°C for 2 d. Vegetative cells were then plated and grown on nutrient agar (NA; Difco) at 50°C for 7 d. The percentage of sporulated spores was determined by phase-contrast microscopy. When sporulation reached 90%, the spore crop was harvested by adding 5 ml of sterile deionized water (DIW), detaching with sterile glass beads, collecting with a pipette, re-suspending in sterile DIW to obtain ~109 spores/ml. The suspension was centrifuged five times at 14,000 × g at 4°C for 10 min. It was then sonicated for 10 min (SM275HT, Crest, ETL Testing Laboratory, Cortland, NY). Heat treatment at 80°C for 15 min was applied to destroy any remaining vegetative cells. The spore suspension was stored at 4°C until used.

Aliquots of 0.2 ml *B. coagulans* spore suspension and 1.8 ml tomato juice (Campbell Soup Company, Camden, NJ) of pH 4.2 were aseptically transferred into a sterile pouch (5 x 2.5 cm, polyethylene bags, 01-002-57, Fisher Scientific) to obtain ~2.5 x 108 CFU/ml. The pouches were then heat sealed using an impulse heat sealer (American International Electric, Whittier, CA), while removing excess air from the pouch. Samples were stored up to 2 h in an ice-water bath (4°C) before treatment.

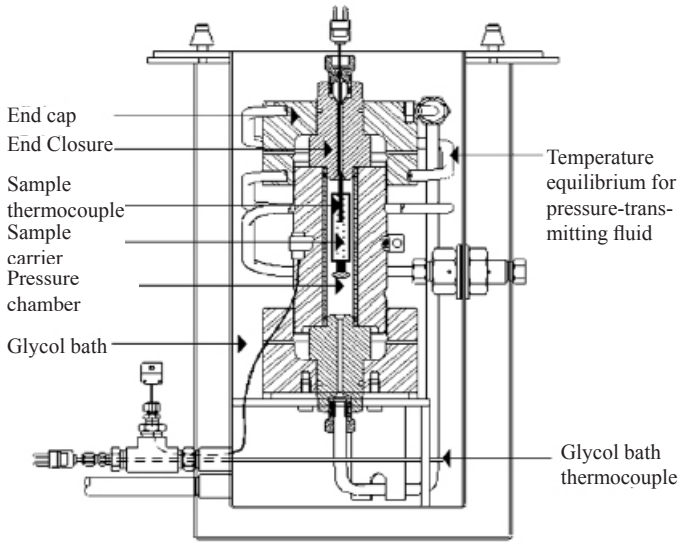


**Figure 1**. Cross section of high pressure microbial kinetic tester (Adapted from: Rajan et al., 2006)

A high pressure microbial kinetic tester (PT-1; Avure Technologies Inc., Kent, WA) was used to treat the samples (Figure 1). The PT-1 unit is equipped with an intensifier (M-340 A, Flow International, Kent, WA) that can generate pressures up to 700 MPa. A 54-ml stainless steel pressure chamber was immersed in a temperature-controlled bath. Propylene glycol (Houghton Safe-620TY, Houghton International Inc., Valley Forge, PA) was used as the heat transfer medium. The temperature of the external glycol bath was set at 100°C (the target process temperature) to minimize any loss during the test. The high pressure processor had a pressurization rate of 14.3 MPa/s, while depressurization occurred within 2 s for all treatments. The sample temperature, bath temperature, and chamber pressure were recorded with a K-type thermocouple sensor (model KMQSS-04OU-7, Omega Engineering, Stamford, CT) and pressure transducer (model 3399 093 006, Tecsis, Frankfurt, Germany) using a data acquisition computer.

Each sample pouch was placed inside a 10-ml polypropylene syringe (model 309604, Becton, Dickinson and Company) sample holder that was wrapped with two layers of insulating material (Sports Tape, CVS® Pharmacy Inc., Woonsocket, RI). The void volume in the syringe was filled with water to ensure that the samples and the surrounding syringe water experience similar thermal response during processing. Insulating syringe further helped minimize the heat exchange between the syringe containing the spore sample and the surrounding glycol bath.

The syringe was then loaded into the pressure chamber and the chamber was sealed. After reaching the predetermined temperature (T2), the pressurization process started. The T2 was estimated using the following relationship:[7]

$$T_2 = T_3 - (CH \bullet \Delta P + \Delta T_H) \qquad (1)$$

where T3 is the desired target temperature (°C), CH is the heat of compression value of water (oC/100 MPa), and ΔP is the process pressure (MPa). ΔTH is the temperature gained by the test sample during loading within the pressure chamber as well as pressurization.

Test samples were treated at various PATP conditions (600 MPa and 100°C for 30, 45, 60, 75, 90, and 120 s). The pressure holding time did not include the pressure come-up time or the depressurization time. After depressurization, the spore suspension was removed from the pressure chamber and cooled in an ice-water bath (4°C) to prevent further inactivation. The samples were enumerated within 3 h after the treatments. All experiments were independently repeated at least three times.

The treated samples were subsequently heat shocked at 80°C for 15 min to kill germinated and sensitized spores occurring during the PATP treatments. After heat shock, 1 ml of the treated suspension was serially diluted in sterilized 0.1% peptone water. The 0.1-ml aliquots of the appropriate dilutions were then spread-plated on duplicate NA plates. The plates were incubated at 37°C for 48 h and the colony survivors were then counted.

## Results and Discussion

The *B. coagulans* spores were inactivated logarithmically by PATP treatment 600 MPa and 100°C (Figure 2). A 3.4 log reduction occurred during the 30 s come-up time. This suggests that the bacteria are sensitive to the initial pressure treatment. A 7.4 log reduction was achieved after 120 s holding time. From these results, a D-value of 30 s was estimated for this treatment by calculating the average linear slope of the survivor curve (Figure 2) using the first-order kinetic equation:

$$Log\ N/N_0 = -t/D \qquad (2)$$

where $N_0$ is the initial spore population and $N$ is the population after a treatment time of t. Undetectable levels were ≤10 CFU/ml. Untreated samples were at the original concentration after heat-shock. Temperature and pressure were regulated throughout the experiment and did not fluctuate significantly (Figure 3). Treatments in which the temperature or pressure fluctuated were discarded and not included in the data. Compared to the literature, *B. coagulans* appears to be more sensitive to combined pressure and heat treatment compared to heat alone.

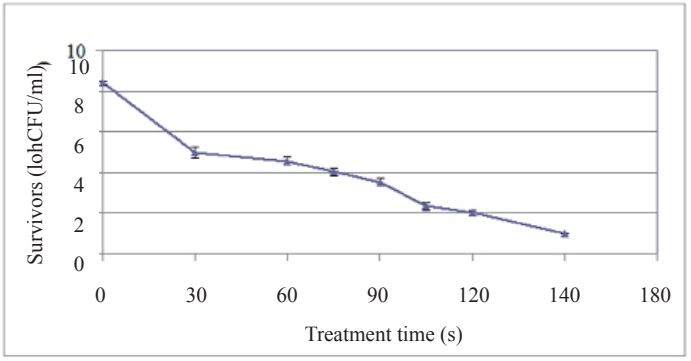


**Figure 2**. Inactivation of *B. coagulans* spores in tomato juice at 600 MPa and 100°C.

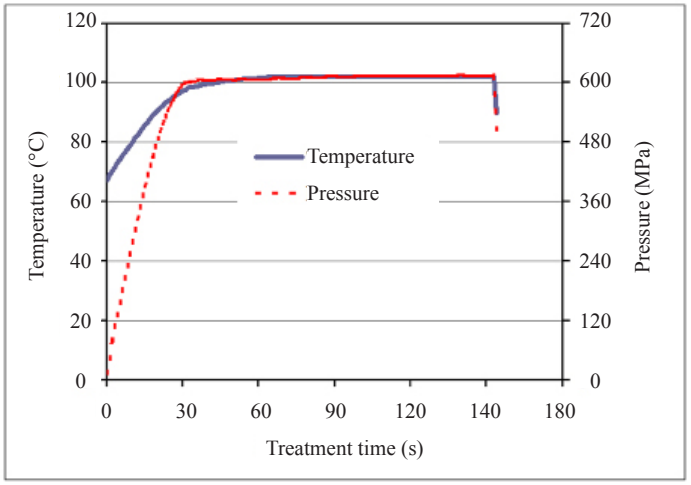


**Figure 3**. Pressure-temperature profile for PATP conditions.

## Conclusions

The D-value for *B. coagulans* at 100°C was estimated to be 30 s. Because the temperature is less severe and D-value is lower (and therefore processing time is less), it can be considered to be an alternative to thermal processing that may produce foods with fresher organoleptic attributes. Studies are currently in progress to estimate combined pressure-thermal resistance kinetic parameters (D and z values) of the 185A strain suspended in tomato juice. It is recommended that the effects of pH and pre-conditioning temperatures on the inactivation of *B. coagulans* in tomato juice be studied further. Finally, the least severe heat and pressure conditions to inactivate the spores and preserve quality attributes should be identified.

## Acknowledgements

Thank you to Drs. Balasubramaniam and Daryaei, both of whom provided support and assistance throughout my research. The research was supported in part by a USDA National Research Initiative Competitive Grant project entitled "Quality of foods processed using selected alternative processing technologies".

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