

Compression heating influence of pressure transmitting fluids on bacteria inactivation during high pressure processing

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Abstract

Compression heating characteristics of different pressure transmitting fluids [three different concentrations (75:25, 50:50, 25:75) of water–glycol mix and sodium benzoate (2%) solutions] and their influence on inactivation of spores of *Bacillus subtilis* in phosphate buffer (0.067 M, pH 7.0) during high pressure processing (HPP) were studied. Experiments were conducted using a pilot scale food processor. Pressure transmitting fluids containing highest percentage of glycol (25:75 water–glycol mix) showed highest temperature increase while 2% sodium benzoate solution showed least temperature increase during high pressure processing. The target pressure, holding time, compressibility, initial temperature, and the rate of heat loss to the surroundings primarily influenced the apparent temperature increase of pressure transmitting fluid in a vessel during HPP. The temperature change was further influenced by the fluid properties such as viscosity, specific heat and thermal conductivity. Use of sodium benzoate solution as pressure-transmitting fluid resulted in highest inactivation of *B. subtilis* spores. Change in pressure transmitting fluid temperature as a result of compression heating and subsequent heat transfer should be considered in inactivation of bacterial spores by HPP.

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1. Introduction

High pressure processing (HPP) is a novel method of food preservation in which elevated pressure is applied in a quasi-instantaneous manner throughout the food (Cheftel, 1995). As the pressure is transmitted instantaneously, the processing time is independent of the sample volume and shape. In contrast to traditional thermal processing, HPP retains the sensory qualities of the food (Hayakawa et al., 1994). HPP can inactivate vegetative bacteria (Cheftel, 1995; Hoover Metrick, Papineau, Farkas, & Knorr, 1989; Ohshima, Ushio, & Koizumi 1993; Smelt, 1998). As with other inactivation treatments, bacterial spores are very resistant to HPP. At ambient temperatures, bacterial spores can survive

pressures above 1000 MPa. A combination of elevated pressure and moderate temperature is needed for inactivation of spores (Cheftel, 1995; Rovere, 1995; Hoover et al., 1989). Seyderhelm and Knorr (1992) reported that the *Bacillus stearothermophilus* spores subjected to 200 MPa at 90 °C for 30 min are reduced by two log₁₀ from an original count of 10⁶. When the temperature was lowered to 80 °C, the same reduction required 30 min at 350 MPa, while at 70 °C, there was little inactivation even after 45 min at 400 MPa. Thus, the temperature plays a crucial role in high-pressure inactivation of spores. Reddy et al. (1999) observed a greater log reduction of *Clostridium botulinum* spores of type E in phosphate buffer when the temperature was increased from 35 to 55 °C at a high pressure of about 827 MPa for 5 min. They obtained a 5-log reduction of type E (Alaska) spores in phosphate buffer after processing at a pressure and temperature combination of 827 MPa and 40 °C for 10 min holding time. Balasubramaniam, Reddy, and Palaniappan (1997) reported that a 3- to 8-log reduction

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of *Bacillus subtilis* spores was obtained at temperatures above 55 °C for a range of pressures (275–827 MPa) with holding times ≥ 5 min.

Pressure-transmitting fluids are used for uniform transfer of pressure to the food. Many of the early laboratory machines were not fabricated from stainless steel and necessitated the use of oils as the pressure medium. Solutions of castor oil, silicone oil, water, sodium benzoate, and glycol are commonly used as pressure-transmitting fluids (Ting, Tremoulet, Hopkins, & Many, 1998). Ability of the pressure transmitting fluid to protect inner vessel surface from corrosion during processing and viscosity of the fluid under pressure are some of the factors involved in the fluid selection. When water is used as a pressure transmitting fluid, it undergoes a 15% decrease in volume at high pressures of around 600 MPa (Farr, 1990). The physical compression results in a temperature increase (2–3 °C per 100 MPa) (Cheftel, 1995). When organic solvents or oils are employed as pressure transmitting fluid, the temperature increase is greater than that of water, due to their higher compressibility, lower thermal conductivity and lower heat capacity (Makita, 1992). Difference between compressibility of the pressure transmitting fluid and the food sample may result in heat transfer between pressure transmitting fluid and food sample. The potential influence of compression heating characteristics of pressure transmitting fluids on microbial inactivation is not investigated. The objective of this study was to compare the apparent temperature increase of selected pressure transmitting fluids during high pressure processing using a pilot scale food processor and study their effect on inactivation of spores of *B. subtilis* in phosphate buffer.

2. Materials and methods

2.1. Preparation of pressure transmitting fluids

Food grade water-based glycol, i.e. Houghto Safe-620TY (HS) (Houghton International Inc., Valley Forge, PA) and Sodium Benzoate (Fisher Scientific, Fair Lawn, NJ) were used for preparing various pressure transmitting fluids. The fluids were selected based on their prior use by the industry. Different concentrations of HS solutions (75, 50 and 25%) were prepared by mixing with de-ionized water (W). The prepared mixtures were denoted as follows: WHS2575 (W=25% and HS=75%), WHS5050 (W=50% and HS=50%) and WHS7525 (W=75% and HS=25%). A 2% sodium benzoate (SB) solution in de-ionized water (W) was prepared and denoted as WSB9802. All four solutions were stored at 4 °C until used.

2.2. Preparation of *B. subtilis* spores

B. subtilis ATCC 6633 spores obtained from the culture laboratory of the Department of Food Science, Ohio State University, Columbus, OH, was used in this study. A stock culture was inoculated into nutrient broth consisting of 5 gm/l Bacto Peptone (DIFCO Labs, Detroit, MI), 3 gm/l Beef Extract (Becton Dickinson, Cokesville, MD), and incubated at 37 °C for 24 h.

A portion of the 24-h culture was maintained on a nutrient slant stored at 4 °C. Another portion of the culture was inoculated into nutrient broth and incubated for 18 h at 37 °C. The 18 h culture was spread-plated on nutrient agar fortified with 500 ppm Bacto-Dextrose (DIFCO Laboratories, Detroit, MI) and 3 ppm Manganese Sulfate (Fisher Chemicals, Fairlawn, NJ) for sporulation. The plates were incubated for 6–8 days at 37 °C. Sporulation was monitored by phase contrast microscopy. After 95–99% sporulation was attained, the plates were stored at 4 °C for 24 h. Flooding the agar surface with 10 ml of cold sterile distilled water and scraping the agar surface with a sterile bent glass rod harvested the spores. The harvested spore suspensions were removed with a Pasteur pipette and sonicated for three, 2-min periods with 20 min intervals between sonication. The spore suspensions were washed six times by centrifugation (at $8000 \times g$ for 20 min at 4 °C) and resuspension in cold sterile distilled water after each centrifugation. After the final centrifugation, the pellets were resuspended again in cold sterile distilled water (to obtain a concentration of 1×10^8 spores/ml) and stored at 4 °C until used.

2.3. Packaging of samples for compression heating studies

About 50 ml of water or potato chunks (27.2 g) and 25 ml water were placed in a high barrier film pouch (CN-530 film, Cryovac, Sealed Air Corp., Duncan, SC) and sealed with a heat sealer (Doughboy Heat Sealer, Dohoy Packaging Machinery Inc., New Richmond, WI). Air was excluded from the package. The sample pouches were placed individually into a slightly larger high barrier pouch (P640B film, Cryovac, Sealed Air Corp., Duncan, SC) and heat-sealed. The samples were subjected to a pressure of 759 MPa, temperature of 30 °C and a holding time of 10 min using a Quintus model QFP-6 high pressure food processor, (Flow Autoclave Systems, Columbus, OH). The initial temperature of the pressure fluid was maintained close to the external water jacket temperature i.e., 30 °C at the start of the cycle to measure the temperature increase as a result of compression. The process time did not include the pressure come-up or de-pressurization times. A Hewlett Packard data acquisition system (HP75000, Series B, model E1301A) supported by a HPVEE Windows compatible software (version 3.1, Hewlett-Packard

Company, Palo Alto, CA) was used for collecting the data. A k-type thermocouple mounted within the test area of the high-pressure processor monitored the temperature of the pressure-transmitting fluid (Fig. 1). Another k-type thermocouple monitored the temperature of the water jacket surrounding the pressure vessel. Since the temperature of the pressure transmitting fluid and the test sample changed during high pressure processing because of compression heating, the temperature of the water jacket was designated as the process temperature. Pressure and temperature transducers used in the study are calibrated on a semi-annual basis through a maintenance program with the equipment manufacturer.

2.4. Packaging and high pressure processing of *B. subtilis*

One millilitre of diluted *B. subtilis* spore mixture (1×10^8 spores/ml) and 9.0 ml of phosphate buffer (0.067 M, pH 7.0) were placed in a sterile high barrier film pouch (CN-530 film, Cryovac, Sealed Air Corp., Duncan, SC) and sealed with a heat sealer. The sealed sample pouches were then placed in a larger high barrier film bag (P640B film, Cryovac, Sealed Air Corp., Duncan, SC) and heat-sealed. Two of these sample pouches were placed inside a larger high barrier film bag (P640B film, Cryovac, Sealed Air Corp., Duncan, SC) and heat-sealed. Tests with the samples containing *B. subtilis* spores were conducted at 827 MPa (827 MPa \approx 120,000 psi) and two temperatures (50 and 70 °C) for various process times (0.01, 3, 5 and 10 min). To maintain isothermal conditions during the cycle, the initial tempera-

ture of the pressure transmitting fluids and the samples were lowered to compensate for the compression heating effects. This was accomplished through a set of preliminary experiments. The processed pouches were held under crushed ice and water at 4 °C until they were opened for enumeration.

The surviving spores in the processed pouches were determined by a pour plate method (AOAC, 1998) using Bacto peptone–beef extract–manganese sulfate–dextrose agar as a medium and incubating the plates at 37 °C for 48 h. Spore count reductions were expressed as the difference of log counts of the sample after treatment (N) and the sample before treatment (control) (N_0). Each value is the average of triplicate samples. Each value is the average of triplicate samples.

2.5. Properties of the pressure transmitting fluids

The thermal conductivity of the fluids was estimated based on the methods by Murakami et al. (1996), Murakami (1997) and Sweat and Haugh (1974). Care was taken to ensure that the convection currents were minimized or totally avoided during the experiments. A Differential Scanning Calorimeter measured the specific heat capacities of the various pressure transmitting fluids. The viscosity of the pressure transmitting fluids at atmospheric pressure was measured by using a Brookfield Viscometer (model: LVDV-II +, Brookfield Engineering Laboratories, Inc., Stoughton, MA). Thermal diffusivity of the various test fluids was calculated using the thermal conductivity, viscosity, and specific heat data at 30 °C. All the experiments were conducted

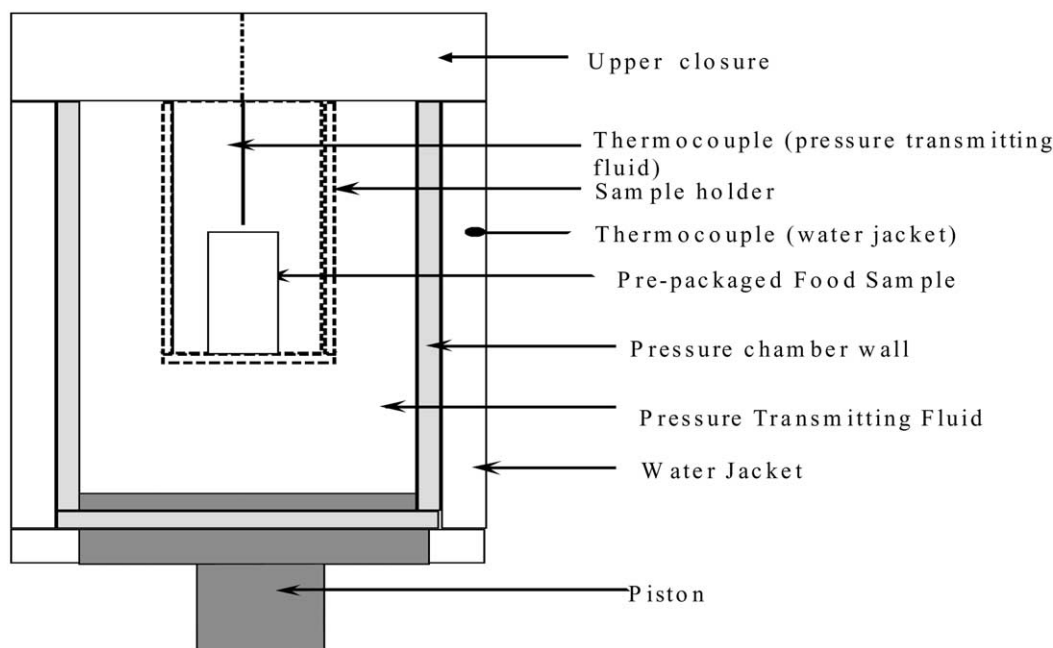


Fig. 1. Schematic diagram of high-pressure chamber for the QFP-6 high-pressure food processor.

at atmospheric pressure conditions. The values at the elevated pressure–temperature process conditions were not determined due to lack of suitable instrumentation.

2.6. Statistical analysis

The data were analyzed using General Linear Model (GLM) procedure of Statistical Package for Social Sciences (ver. 8, SPSS Inc., Chicago, IL). Analysis was based on at a significance level (α) of 0.05.

3. Results and discussion

3.1. Influence of pressure transmitting fluids on compression heating

All compressible substances change temperature because of physical compression during high pressure processing. This is very predictable thermodynamic effect. For example, the thermodynamic properties of water under pressure were well documented and the data are available from International Association for the Properties of Water and Steam (IAPWS). A software implementation of IAPWS work can be obtained from the National Institute of Standards and Technology (NIST) (Harvey, Peskin, & Klein, 1996).

The magnitude of the temperature change depends mainly on the compressibility of the substance and its specific heat. Water, a major constituent of food, is compressed up to 15% volume at pressure above 600 MPa. As the rate of decrease in the volume of the fluid approaches towards a zero, then the temperature of the fluid starts to increase rapidly due to the work done in compressing the fluid and the resistance offered by the fluid for further compression. Once the desired pressure is reached, the work on further compressing the fluid is stopped and energy is spent only on maintaining the pressure for the length of the processing time. During this time, heat transfer takes place between the pressure transmitting fluid, sample, and the external environment (through the walls of the pressure chamber). The time at which the desired pressure is reached depends in part on the compressibility of the pressure transmitting fluid and food samples used, the sample to fluid ratio in the pressure chamber and high-pressure equipment design parameters such as the type of the pump used.

The effect of compression heating on pressure transmitting fluids temperature history is illustrated in Figs. 2 and 3. As shown, the glycol-based pressure transmitting fluids experienced a significantly different processing history from the one in sodium benzoate water, even though all runs were conducted at an identical process conditions. The temperature history achieved reflects the experimental conditions used, and can be varied by modifying the heat-transfer characteristics of the

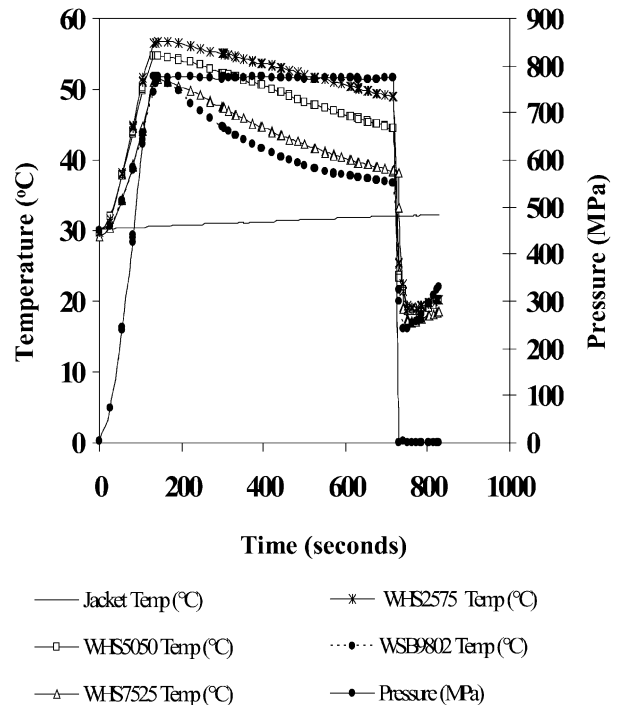


Fig. 2. Pressure and sample temperature profiles obtained for different pressure fluids without any compensation for initial temperature (30 °C).

high-pressure vessel. No attempt was made to measure temperature of the sample inside the pouch under pressure due to lack of instrumentation. The temperature profiles obtained for pressure transmitting fluids represent apparent temperature increase (i.e., difference between true temperature increase due to compression heating and heat loss to the surroundings). Any variation in heat transfer characteristics of the pressure vessel (such as area/volume ratio of the pressure chamber, location of the temperature sensor within the pressure vessel and insulation properties of the pressure chamber material) likely yield different temperature profile.

Heat transfer from the fluid to the chamber under pressurization can mimic a refrigeration cycle (Ting et al., 1998). During pressure-come up, about 26–27 °C increase over the initial temperature (30 °C) was observed for WHS2575 fluid when processed at a pressure of 759 MPa, and holding time of 10 min at 30 °C (Table 1). The increases were 24–25 and 21–22 °C respectively for WHS5050 and WHS7525 fluids. WSB9802 fluid had an increase of about 22 °C over the initial temperature of 30 °C. Among the water–glycol solutions tested, compression heating was highest for WHS2575 fluid and least for WHS7525 fluid. WHS7525 and WSB9802 exhibited the least temperature increase and were less viscous. They had highest cooling effect during pressure processing (Tables 1 and 2). Once the target pressure is reached, the temperature increase gained by the pressure fluid could be lost to the product

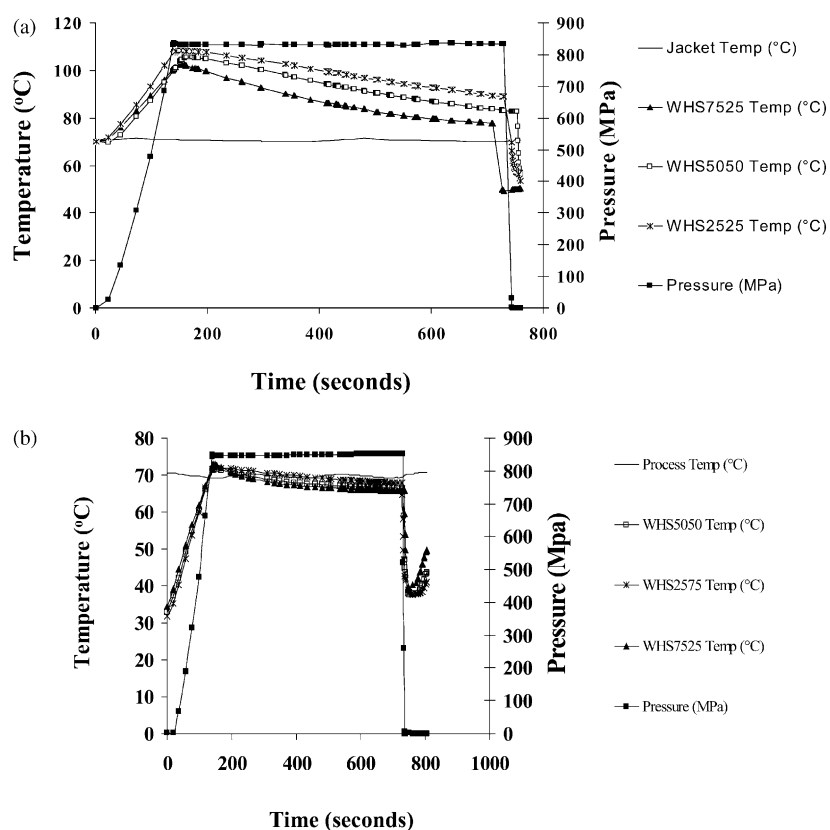


Fig. 3. Pressure and sample temperature profiles obtained for different pressure fluids (a) without any initial temperature compensation (70 °C) and (b) with initial temperature compensation (70 °C).

Table 1

Compression heating characteristics of various pressure transmitting fluids at a pressure of 759 MPa and a holding time of 10 min without any temperature compensation

Fluid	Jacket temperature (°C)	Pressure fluid temperature (°C)			Temperature drop during processing (°C) ^a	Time to reach max. temp. after reaching target pressure (s)
		Initial	Maximum	Final		
WHS7525	30.1±0.1	29.5±0.2	51.6±0.3	38.8±0.7	12.8±0.5	14.3±2.6
WHS5050	29.9±0.6	30.0±0.1	54.5±0.7	44.8±0.3	9.7±1.0	24.0±5.7
WHS2575	30.0±0.0	29.7±0.1	56.7±0.2	48.7±1.1	8.0±1.3	36.0±7.7
WSB9802	30.4±0.3	30.5±1.1	52.6±1.5	36.7±0.5	15.8±1.0	7.0±2.9

^a During process holding time.

and the environment through the walls of the high-pressure chamber during processing. Temperature decreased at a rate of 0.8 °C/min for WHS2575 fluid during the holding time of 10 min at a constant pressure. The decrease rate was 1.6 °C/min for WSB9802 (Table 1). This difference may be attributed to the differences in fluid viscosities. Further, the difference in fluid thermal conductivity and specific heat may have caused the temperature variation. Upon decompression, the temperature of the content falls below the initial starting temperature of the test. This is essentially similar to a refrigeration cycle at work in which the refrigerant is the content of the pressure chamber.

It is worth noting though temperature curve readily followed pressure curve during pressure come-up period, different pressure-transmitting fluids took different time (Table 1) to reach maximum temperature upon pressurization. If the solution contains more glycol, it took longer (up to 43 s) to reach the maximum temperature. Otero, Molina-Garcia, and San (2000) also reported temperature delays during expansion. Further research is necessary to significance of this phenomenon.

Figs. 2 and 3 also illustrate the constraints in reporting the process temperature at pressure. For example, when no temperature compensation provided and

process temperature and initial temperature of the pressure transmitting fluid and the test samples are the same (70 °C), the temperature increase in the pressure fluids ranged from 33 to 37 °C above the desired target temperature (Fig. 3a). On the other hand, if the temperature of the pressure transmitting fluids and the sample are lowered to account for respective compression heating effects at target pressure and temperature, the final temperature at pressure are very close to the desired process temperature (Table 2). The initial temperature of the pressure transmitting fluid and the sample can be selected based on factors, such as the type of material, process holding time, and target pressure, pressure fluid-sample load within the chamber, and chamber design. The longer the holding time, the higher the initial pressurizing medium temperature should be, to compensate for the steady drop through out the hold cycle of the process run. It would be desirable to report the beginning and end temperature of the pressure transmitting fluid under pressure as well as the initial and final temperatures at atmospheric pressure (Balasubramaniam & Ting, 2001).

3.2. Influence of pressure transmitting fluids on spores inactivation

Irrespective of pressure transmitting fluids used, inactivation of *B. subtilis* spores was minimal (<1 log) (Fig. 4) at 827 MPa and 50 °C. About 6.6 log unit reduction of spores was obtained at 827 MPa and 70 °C combinations for a process time of 10 min for WHS7525 fluid. For other two fluids (WHS2575 and WHS5050), about 5–6 log unit reductions were observed. A maximum inactivation of about 8-log unit reduction of *B. subtilis* spores was observed for WSB9802 fluid.

If the pressure-medium fluid exhibits high compression heating, the inactivation data could include unintended thermal effects (Ting, Balasubramaniam, & Raghubeer, 2002). Under ideal adiabatic conditions (no

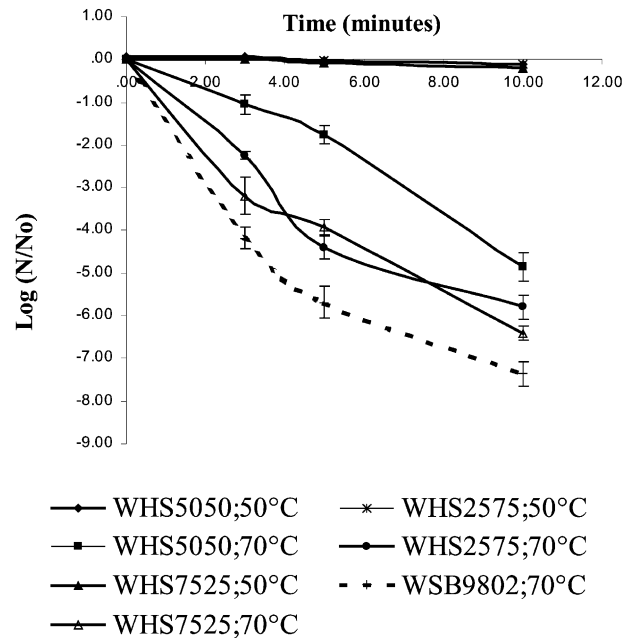


Fig. 4. Inactivation of *Bacillus subtilis* spores at a pressure of 827 MPa and temperatures of 50 and 70 °C using different pressure transmitting fluids.

heat loss to the surroundings), for same initial fluid temperature and other constant process parameters, solution containing highest percentage of glycol (i.e., WHS2575 fluid with highest compression heating rates) may produce highest inactivation rate. Contrary to expectations, nearly 2-log₁₀ greater inactivation values were obtained when WSB9802 solution was used as the pressure transmitting fluid. This may be due to use of higher initial temperature by this fluid and the difference in the thermal properties of the fluids. At higher pressures, the thermal conductivity of a solution is higher than its thermal conductivity value at atmospheric pressure (Denys & Hendrickx, 1999). The thermal diffusivity of WSB9802 solution at a temperature of 30 °C and atmospheric pressure was nearly 1.5 times of the thermal diffusivity value of WHS2575 solution at the same conditions (Table 3). Glycol based fluids with lower thermal diffusivity; it may take longer time for the heat to reach the sample. A slight increase in the hold-

Table 2

Compression heating in the different pressure transmitting fluids at a pressure of 827 MPa and initial sample temperature of 70 °C for a holding time of 10 min

Fluids	Jacket temperature (°C)	Pressure fluid temperature (°C)			Temp. drop (°C)
		Initial	Max.	Final	
<i>(a) With no initial temperature compensation</i>					
WHS7525	70.2±0.3	69.9±0.7	102.8±1.3	78.1±0.5	24.7±1.2
WHS5050	70.4±0.4	69.0±0.8	104.8±0.7	82.7±0.9	22.1±0.9
WHS2575	70.8±0.3	70.3±1.1	107.4±1.81	89.6±1.9	17.8±1.8
<i>(b) With initial temperature compensation</i>					
WHS7525	70.0±0.2	34.5±1.8	72.9±1.5	65.8±0.5	7.2±0.8
WHS5050	70.1±0.2	32.8±1.5	71.7±0.8	66.4±0.2	5.3±0.4
WHS2575	70.0±0.5	31.9±1.1	72.0±0.9	67.8±0.3	4.2±0.5

Table 3

Thermal properties of the various pressure transmitting fluids determined at 30 °C and 0.1 MPa (1 atmospheric pressure)

Fluids	Specific heat, Cp (kJ/kg/K)	Viscosity, μ_f^a (10 ⁶ Ns/m ²)	Density, (kg/m ³)	Thermal conductivity, k (W/m/K)	Thermal diffusivity, ρ (m ² /s)
WHS2575	3.20	30 800	944	0.39	1.3×10 ⁻⁷
WHS5050	3.25	19 710	957	0.50	1.6×10 ⁻⁷
WHS7525	3.10	5260	983	0.56	1.84×10 ⁻⁷
WSB9802*	3.23	855	1011	0.61	1.87×10 ⁻⁷

^a Viscosity and thermal conductivity values were assumed to be equal to that of water.

ing time may cause the desired amount of heat to reach the sample and result in an identical log reduction of the spores obtained for WSB9802 solution. At higher pressures (827 MPa) and temperatures (70 °C) conditions, the thermal diffusivity values of the fluids to be higher than that shown in Table 3.

Meyer, Cooper, Know, and Leieveld (2000) reported a similar observation on the role of pressure transmitting fluids on microbial inactivation during sterilization process and stressed the importance of considering difference in thermal properties of pressure transmitting fluids and the product for reproducible microbiological sterilization experiments. It is important to use the same type of pressure transmitting fluid for both laboratory scale and industrial scale production for microbiological testing. A new type of pressure transmitting solution may change the final temperature, because it may have different adiabatic compression heating properties. It would be further desirable to match compression-heating characteristics of pressure fluid and the product to minimize the temperature gradient between them.

The results also demonstrate the importance of controlling temperature during processing a microbial sample during high pressure processing especially those involving sterilization studies. High-pressure research community is currently experimenting with various approaches including use of an internal heater (as the case in the present study) and/or use of insulated pressure chambers. Since many of the earlier studies did not consider the influence of thermal effects during HPP on microbial inactivation, for similar process conditions, often-conflicting microbial inactivation data were reported. The potential process non-uniformity may also contribute to tailing effect. Knowledge of the temperature distribution will likely help other laboratories to reproduce test conditions on different test equipment.

4. Conclusions

The apparent temperature increase in pressure transmitting fluid during HPP is influenced by the target pressure and holding time, product compressibility, and initial temperature and the rate of heat loss to the surroundings. Among the fluids tested, pressure-transmitting fluid containing the highest percentage of glycol, i.e. the WSB2575 solution showed the greatest compression heating effect. WSB9802 solution, containing the highest percentage of water resulted in a temperature increase of about 22 °C due to compression heating. This solution had the highest thermal diffusivity and the least viscosity, which may have contributed to increased inactivation of *B. subtilis* spores, i.e., > 2 log unit reduction compared to WSB-based fluids. Change in pressure transmitting fluid temperature as a result of compression heating and subsequent heat transfer

should be considered during HPP microbial inactivation studies involving bacterial spores.

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