Use of High Hydrostatic Pressure and Irradiation To Eliminate Clostridium sporogenes Spores in Chicken Breast

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ABSTRACT

High pressure has been studied for its usefulness in reducing microbial contaminants in foods. We sought to determine whether this technology could be used in combination with irradiation to develop shelf-stable products. We first determined the optimal pressure, temperature, and time conditions that would result in maximum reduction of Clostridium sporogenes spores in fresh chicken. At ambient temperature, a pressure of 6,800 atm for up to 60 min resulted in a 5-log-unit reduction. Heating the samples during pressurization at 80°C for 20 min resulted in the lowest number of survivors compared to samples that were heated and pressurized for only 1 and 10 min. Further, irradiation at a medium dose (3.0 kGy) before and after pressurization at 6,800 atm and 80°C for 1, 10, and 20 min revealed no significant differences in spore counts between samples that were pressurized and then irradiated or vice-versa. We then examined the effect of high pressure in lowering the irradiation dose necessary to eliminate all spores. The irradiation D value of C. sporogenes spores was calculated to be 4.1 kGy. Samples were then irradiated at various doses followed by pressurization at 6,800 atm at 80°C for 20 min. The irradiation D value was lowered to approximately 2 kGy, indicating that a combination of high hydrostatic pressure and irradiation can be used to produce chicken with an extended shelf life without the use of high irradiation doses.

Key words: Clostridium sporogenes, high pressure, irradiation, chicken

High pressure provides a viable alternative to thermal processing as it does not markedly alter the color, flavor, and nutritional content of most foods (1). High pressure is "isostatic and instantaneous," meaning that it is transmitted evenly on all surfaces (17, 18). It is considered a "cold process," since most foods can be pressurized at ambient temperatures (1, 4, 18). This process is influenced by various factors such as the amount of pressure applied, the duration of the compression, the depressurization rate, temperature, product pH, water activity, and salt concentration (18).

The effect of high hydrostatic pressure on bacterial cells is primarily on the cell membrane. Increased permeability of the cell membrane following high pressure results in cell death (7). Shigehisa et al. (17) found that pressures above 44,000 lb/in² (2,994 atm, ca. 303 MPa) inactivated Campylobacter jejuni and Yersinia enterocolitica, and that Escherichia coli and Candida utilis were inactivated at pressures above 58,000 lb/in² (3,946 atm, ca. 400 MPa). However, Staphylococcus aureus and Streptococcus faecalis were only inactivated at pressures of about 88,000 lb/in² (5,988 atm, ca. 607 MPa). These results along with others (1) suggest that gram-negative bacteria are inactivated at lower pressures than gram-positive microorganisms. Bacterial spores are considerably more resistant to high pressures, with some studies having found that endospores of Bacillus and Clostridium can survive pressures above 176,000 atm (ca. 178 MPa) (6, 14, 20).

Another process that is useful in eliminating foodborne pathogens and is gaining renewed interest is irradiation. The use of ionizing radiation in eliminating foodborne pathogens and extending the shelf life of food products has been firmly established by numerous studies (12, 13). Almost all pathogens in their vegetative forms are highly sensitive to irradiation, and a low dose of 3.0 kGy is sufficient to eliminate them (13). However, as with high pressure, Clostridium and Bacillus spores are resistant to low doses of irradiation. For instance, C. botulinum spores are known to exhibit great variability in radiation resistance, requiring doses of 19 to 61 kGy for their inactivation (2). Combining processes like irradiation and high pressure could be used to reduce the treatment required of any one process if used alone. In doing so, any nutritional or sensory losses could be minimized.

The purpose of this study was to determine whether high pressure could be used to lower the irradiation dose required to eliminate C. sporogenes spores in chicken.

MATERIALS AND METHODS

Bacterial culture and spore crop preparation
Clostridium sporogenes ATCC 7955 was obtained from the American Type Culture Collection at Rockville, MD. The culture was maintained in cooked meat medium (Difco Laboratories, Detroit, MI) at −20°C until needed. Destruction of vegetative cells...
prior to inoculation was achieved by heating the culture in an 80°C waterbath (Model 730, Fisher Scientific, Pittsburgh, PA) for 10 min. One milliliter of the culture was then inoculated into 150 ml of sporulation medium, composed of 3% Trypticase soy broth (Becton Dickinson Microbiology Systems, Cockeysville, MD), 0.1% yeast extract (Difco), and 1% (NH₄)₂SO₄ (Fisher Scientific Co., Pittsburgh, PA). The culture was incubated anaerobically in a Gas Pak jar (Fisher Scientific) at 37°C for 48 h for maximum spore production. The samples were centrifuged twice in a Beckman J2-21 centrifuge at 9,253 × g (Beckman Instruments, Inc., Palo Alto, CA) for 20 min and then diluted with a 0.1% peptone solution to obtain a concentration of approx. 1 × 10⁶ spores per ml. The spore count was determined by pour plating in brain heart infusion agar. The prepared spore crop was stored at 4°C until used.

Sample preparation and inoculation

Fresh boneless chicken breast samples were purchased from a local retail store in Ames, IA and stored overnight at 4°C until use. The chicken breasts were cut into 25-g portions, inoculated with C. sporogenes spores, and placed into high barrier pouches (Curlton® Grade 861, Curwood Inc., Oshkosh, WI) having O₂ permeability of 3 to 4 cm³/100 in² (645.16 cm²)/24 h at 22.8°C and 0 RH. The samples were inoculated with 0.1 ml of a culture of 1 × 10⁶ spores per ml by injecting the suspension with a sterile syringe (Becton Dickinson) into the center of the samples to yield a final concentration of approximately 1 × 10⁸ spores per g of meat. The sample bags were vacuum sealed (Fresh Vac. Model A300, CVP Systems Inc., Downers Grove, IL), packaged in ice, and shipped overnight to ABB Autoclave Systems Inc. (Columbus, OH) for pressurization.

High-pressure treatment

The samples were pressurized using the QUINTUS® Food Research Press (ABB Autoclave Systems, Inc., Columbus, OH), capable of operating at 900 MPa, with chamber dimensions of 90 by 225 mm. The samples were submerged in mineral oil, which acted as the hydrostatic pressurization medium. For those samples to be subjected to heating and pressure, the machine was heated to a temperature of 80°C before the samples were introduced into the chamber. The temperature increase due to the adiabatic heating effect during the pressure increase was on average 20°C.

Determination of the optimal pressure-time-temperature combination

After treatment the samples were shipped back overnight to our laboratory at Iowa State University where they were further analyzed for the surviving spore population. The first part of the study involved exposing samples to pressures of 4,100, 5,400, 6,800, and 8,200 atm (ca. 415, 547, 689, and 831 MPa) for 1, 5, 30, or 60 min. Inoculated but unpressurized sample was taken as control. In the second part of the study, inoculated samples were pressurized at 6,800 atm (ca. 689 MPa) and at 80°C for 1, 10, and 20 min. The heating temperature of 80°C was determined from an earlier study by Sarathchandra et al. (15). All the experiments were performed in duplicate.

Following pressure treatment, the 25-g samples were homogenized for 2 min with 225 ml of 0.1% peptone solution in a stomacher (Lab-Blender 400, Tekmar®, Cincinnati, OH). The homogenized samples were serially diluted, pour-plated in duplicate in brain heart infusion agar (BHIA) (Difco) and incubated anaerobically in anaerobic jars at 37°C for 48 h. Five milliliters of the sample homogenate was heated in a waterbath at 80°C for 10 min to inactivate spores that had germinated due to pressure application. The samples were then plated and the colonies formed were considered to represent the surviving spores.

Irradiation treatment

Samples were irradiated with a MeV CIRCE III Linear Electron Accelerator (MeV Industrie S. A., Jouy-en-Josas, Cedex, France) located at the Linear Accelerator Facility at Iowa State University. The samples were irradiated at room temperature by an electron beam at a medium dose of 3 kGy or doses ranging from 2 to 10 kGy for the D-value determinations. A dose of 3 kGy was used in this study as this is the maximum dose allowed for irradiation of fresh and frozen chicken in the US. The dose levels were produced by varying both the power level and the conveyor speed (short or long exposure time). Alanine pellets placed on the top and bottom of the samples were used to measure the absorbed dose by an electron paramagnetic resonance (EPR) instrument (Bruker Instrument, Inc., Billerica, MA). The actual absorbed doses were calculated as the arithmetic mean of the top and bottom doses for each run.

Combination of high pressure and irradiation treatments on C. sporogenes spores

In the first part of the study, inoculated samples were exposed to irradiation at various doses. These were useful in calculating the irradiation D values without pressurization. Next, samples were first irradiated at 3 kGy followed by pressurization at 6,800 atm (ca. 689 MPa) and at 80°C for 1, 10, and 20 min. Unirradiated and unpressurized samples were taken as controls. Thirdly, samples were first pressurized at 6,800 atm at 80°C for 1, 10, and 20 min followed by irradiation at 3 kGy to determine the effect of high pressure prior to irradiation. Lastly, the samples were first irradiated at 2, 4, or 6 kGy followed by pressurization at 6,800 atm at 80°C for 20 min. The last study was useful in determining D values when samples were irradiated at various doses followed by a constant pressurization condition. Following the pressure and irradiation treatments, the samples were enumerated for the surviving spores as described before. All the experiments were performed in triplicate.

Statistical analyses

All microbiological data was analyzed by using the Statistical Analysis System (SAS Institute Inc., Cary, NC, 1986). The analysis of variance (ANOVA) procedure and Tukey’s test of difference were used to detect significant differences between various experimental treatments. The D values were calculated as the arithmetic mean of the top and bottom of the slope obtained by using linear regression analyses of the data.

RESULTS AND DISCUSSION

Optimal pressure-time-temperature combinations

The optimal pressure conditions that were most effective in killing C. sporogenes spores inoculated in chicken meat were determined. An approximately 5-log-unit reduction in viable spores was observed following pressurization at 6,800 atm for 5, 30, and 60 min (Fig. 1). Upon examining the effects of pressure on vegetative cells, Shigehisa et al. (17) found that most microorganisms are completely inactivated at pressures well below 100,000 lb/in² (6,800 atm). However, spores appear to be more resistant to pressure and other environmental stresses that are normally effective on vegetative cells. The present study agrees, since although a pressure of 6,800 atm reduced the viable spore count, a pressure of 8,200 atm was still insufficient to completely eliminate the spores. Sule et al. (14) found that Bacillus and...
Clostridium spores could withstand pressures as high as 117,400 lb/in² (8,000 atm), while Larson et al. (9) found that not all spores of Bacillus subtilis were killed following pressurization at 176,400 lb/in² (12,000 atm) for 12 h.

Figure 1 shows that a decrease in viable spore count was directly proportional to increase in pressure, but only up to a certain level, in this case, 6,800 atm. In addition, a pressure higher than 6,800 atm was ineffective in reducing the spore count. A probable reason for the results of our study is that spore stimulation and germination occurred at less than 6,800 atm and these germinated cells were killed at 6,800 atm, leading to a maximum reduction in spore count at the same pressure. A small fraction of the spore population may have remained highly resistant, capable of withstanding a pressure of even 8,200 atm. An investigation by Mallidis and Drizou (11) on the effect of simultaneous heat and pressure on bacterial spores found that the spore population was heterogeneous with regard to its sensitivity to heat and pressure. Although a large fraction of the spore population was sensitive to heat, there remained a small fraction that appeared to be unaffected. This smaller fraction often survived the combined treatments of heat and pressure. Thus, it is possible that pressurization, even at high doses, results in a small percentage of survivors.

Since a pressure of 6,800 atm showed maximum reduction in viable spore count, it was used for subsequent experiments. The effect of a combination of 6,800 atm pressure and 80°C temperature for a duration of 1, 10, and 20 min was then examined. Figure 2 indicates that application of a constant pressure and temperature (6,800 atm at 80°C) for 20 min was most effective with a significant reduction in spore number. These results indicate that a combination of heat and pressure was more effective in spore destruction than pressure alone.

Effect of pressure and irradiation

The D value calculated after irradiation at various doses at ambient temperature in chicken was 4.1 kGy (Fig. 3). Losty et al. (10) obtained a D value of 2.2 kGy for C. sporogenes PA 3679/S2, and Shamsuzzaman (16) obtained D values ranging from 3.04 to 3.52 for C. sporogenes irradiated in phosphate buffer. One of the reasons for the higher D values in our study is the difference in the suspending media. In the present study, C. sporogenes spores were inoculated into chicken samples, a relatively rich proteinaceous medium that may have had more protective effect on the spores.
when compared to phosphate buffer used in the earlier studies.

There were no significant differences in the number of surviving spores between samples that were first irradiated and then pressurized, or vice-versa (Fig. 4). However, a significant difference was noted between samples exposed to combined treatments and those that were only irradiated (Fig. 4). Clouston and Wills (3) reported that exposure to hydrostatic pressure caused an increase in the sensitivity of *Bacillus pumilis* spores to radiation; however, the results from the present study indicate that pressurizing samples prior to irradiation did not increase the sensitivity of the clostridial spores to the latter. The same can also be said for samples that were irradiated and then pressurized. One reason for this may be that the surviving population of spores were "superdormant," and were therefore extremely resistant to both high pressure and irradiation. Gould (5) reported that "superdormant" spores are dormant towards germination by pressure, and therefore these spores are most likely also resistant to germination by radiation.

A D value of 2.0 kGy was calculated for samples exposed to irradiation at various doses followed by pressurization at 6,800 atm at 80°C for 20 min (Fig. 5). This is approximately one-half the D value of samples that were only irradiated but not pressurized (4.1 kGy). Very limited data are available in the literature where a combination of high hydrostatic pressure and irradiation has been applied in food samples to eliminate bacterial spores. As the pressure treatment in our study was accompanied by heating the samples to 80°C, the results were compared to other thermoderation studies. Kempe (8) found that pre-irradiation inactivation of *C. botulinum* 62A spores with gamma rays sensitized the spores to a subsequent heat inactivation. Additionally, Thayer and Boyd (19) found that irradiation of *Salmonella typhimurium* at 0.90 kGy followed by heating at 60°C for 3 min in chicken meat resulted in about an 8-log-unit decrease in the number of microorganisms compared with a 6-log-unit decrease for samples that were heated and then irradiated.

Results from the present study show a significant decrease in D value when samples were exposed to irradiation prior to pressurization compared to irradiation alone. These results suggest that irradiation before treatment with high pressure sensitized the samples to subsequent pressurization and heating. In conclusion, the results of our study indicate that a combination of lower doses of irradiation and high pressure is more useful in eliminating clostridial spores than application of any one process.

**REFERENCES**