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Accelerated inactivation of *Geobacillus stearothermophilus* spores by ohmic heating

Romel Somavat [a], Hussein M.H. Mohamed [b], Yoon-Kyung Chung [c], Ahmed E. Yousef [d], Sudhir K. Sastry [e,*]

[a] Abbott Nutrition, 3300 Stelzer Road, Columbus, OH 43219, USA
[b] Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt
[c] Hankyong National University, Anseong, Republic of Korea
[d] Department of Food Science and Technology, The Ohio State University, OH, USA
[e] Department of Food, Agricultural and Biological Engineering, The Ohio State University, OH, USA

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ABSTRACT

Until recently, ohmic heating was commonly thought to kill microorganisms through a thermal effect. However a growing body of evidence suggests that non-thermal effects may occur. Our aim was to determine the kinetics of inactivation of *Geobacillus stearothermophilus* spores (ATCC 7953) under ohmic and conventional heating using a specially constructed test chamber with capillary sized cells to eliminate potential sources of error and ensure that identical thermal histories were experienced both by conventionally and ohmically heated samples. Ohmic treatments at frequencies of 60 Hz and 10 kHz were compared with conventional heating at 121, 125 and 130 °C for four different holding times. Both ohmic treatments showed a general trend of accelerated spore inactivation. It is hypothesized that vibration of polar dipicolinic acid molecules (DPA) and spore proteins to electric fields at high temperature conditions may result in the accelerated inactivation.

1. Introduction

Little literature exists regarding the non-thermal effect of electricity on bacterial spores during ohmic heating. In the absence of adequate understanding of spore inactivation kinetics, the method and conditions for ohmic sterilization are typically selected on the basis of existing purely thermal kinetic data (Sastry and Barach, 2000; Iciek et al., 2006). New methods are needed for efficient bio-validation of ohmic heating in order to realize the full potential of the process. If additional non-thermal effects of electricity exist, it may be possible to reduce process severity, potentially improving quality.

Past studies on non-thermal effects of electricity have investigated microorganisms such as yeast, vegetative bacterial cells and bacterial spores. Results reflect gradually evolving, yet inadequate understanding of non-thermal lethality. Palaniappan et al. (1990) and Palaniappan and Sastry (1991) suggested that the destruction of microorganisms during ohmic heating was principally because of thermal effects. However, a reduction in thermal requirement for inactivation of microorganisms was observed at sublethal electrical treatments by Palaniappan and Sastry (1992). Sublethal electrical treatments have also shown a decrease in lag period and modification of metabolic properties during fermentation of lactic acid bacteria (Cho et al., 1996).

Cho et al. (2000) compared the effects of ohmic heating with conventional heating on inactivation kinetics of *Bacillus subtilis* at different temperatures and concluded that the spores heated at 97.2 °C showed a significant reduction in *D*-value under ohmic treatment. However the *Z*-value for the two different methods was not statistically different. A synergistic effect of heat and electrolysis under the influence of low amperage direct electric current on *Saccharomyces cerevisiae* was suggested by Guillou and El Murr (2002). The study explored effects of amperage, temperature, pH and ionic strength. *D*-values were found to be an inverse function of input current density. The above two studies used ohmic devices which were considerably larger than the capillary tube sized sample containers used by conventional microbiologists, and hence lacked precision in the control of experimental errors.

Designs of ohmic devices for microbial studies have remained practically the same since the time when Palaniappan et al. (1990) reviewed the literature on the effect of electricity on microorganisms. They concluded that the data available on non-thermal effects of electricity was limited and inconclusive due to inadequate matching of time temperature histories between electric field treatments and thermal controls. Factors contributing to variability in results may be broadly categorized into two groups: engineering design implications and microbiological constraints. We review these in some detail below: although lengthy, they help justify the design of test chamber that follows.

* Corresponding author. Present address: Department of Food, Agricultural and Biological Engineering, The Ohio State University, 206 Agricultural Engineering Building, 590 Woody Hayes Drive, Columbus, OH 43210, USA. Tel.: +1 614 292 3508; fax: +1 614 292 9448.
E-mail address: sastry.2@osu.edu (S.K. Sastry).

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1.1. Engineering design implications

The literature reveals several logistical and technical constraints relative to the design of ohmic heaters used for studying thermal inactivation kinetics. These factors can either individually influence results or act synergistically.

(a) The size of sample holders used in most ohmic studies has been greater than those generally used for conventional heating treatments. For example, Guillou and El Murr (2002) used an ohmic heater of capacity of 9.2 ml while Cho et al. (2000) used a 100 ml vessel. These vessels have higher possibilities for spatial temperature or cell concentration variations than capillary tubes or tiny thermal canisters used for conventional thermal death time studies. For example, Iciek et al. (2006) used glass capillary tubes of about 100 μl volumes to study inactivation kinetics of *Bacillus stearothermophilus* under conventional thermal processing conditions.
(b) The action of convection currents in fluid media can influence uniformity of the temperature profile inside an ohmic heater or treatment chamber.
(c) Thermal stratification of hot and cold parts of the fluid can occur due to gravity.
(d) Temperature difference between the treatment chamber and surroundings in both conventional and ohmic heating can result in different microbial inactivation rates between the geometric center and the walls of the heater. In ohmically heated samples, internal temperatures are often higher than (unheated) surroundings; for conventional heating, the exterior region may heat faster than the interior of samples. Temperature gradients are minimized during conventional treatments by use of small samples in capillaries. The elimination of temperature gradients is critical in attainment of uniform heating treatments.
(e) Hot or cold spot formation due to one or more of the above reasons acting together.
(f) Temperature histories during ohmic and conventional heating are fundamentally different. Under conventional heating, sample temperature exponentially approaches heating medium temperatures; under ohmic heating, heating often accelerates as temperature increases, since electrical conductivity typically increases with temperature. The challenge for microbiological comparison is to ensure that both histories are identical.

1.2. Microbiological constraints and requirements

(a) Time–temperature histories of all associated processes should ideally match. Variation in factors like come-up time, starting temperature, preparation time and sampling time would result in increased variation in results.
(b) Ideally, immediate cooling should be accomplished at the end of the holding time to ensure that the sample attains a temperature below its optimum range of activity. This will prevent post-treatment activation in case the study is done on spores.
(c) Study of thermo-resistant microorganisms may require treatment temperatures above 100 °C. In such conditions both the treatment as well as cooling should be done under pressurized conditions.
(d) Microorganisms can survive in significant numbers in microcrevices formed around seals (o-rings or rubber gaskets) or on the sides of electrodes, influencing the final enumeration. These parts generally experience a lower temperature than regions between the cross-sectional area of electrodes.

The presence of one or more of these factors may result in errors and explain some of the contrasting results present in the literature. Hence, new and improved experimental methods are needed to compare conventional and ohmic processes.

Our study was aimed at comparing microbial inactivation kinetics under ohmic (using two different frequencies) and conventional heating. Our objectives were:

(1) Development of ohmic and conventional treatment capillary sample cells and an experimental system to enable a legitimate comparison of conventional and ohmic heating on bacterial inactivation.
(2) To explore the effects of ohmic heating frequency (10 kHz and 60 Hz) on inactivation kinetics of *Geobacillus stearothermophilus* (ATCC 7953) spores in comparison to conventional heating.

2. Materials and methods

2.1. System design

Our test device was designed to eliminate the potential sources of error from previous studies. To minimize or eliminate spatial temperature gradients, both conventional and ohmic test cells were made from the same basic capillary cell (construction details follow). The cells were contained entirely within a larger ohmic test chamber filled with a nearly isoconductive solution (0.78% NaCl) optimized to ensure the appropriate heating regimes. This ensured that the test chamber fluid and the samples (within cells) heated at nearly equal rates, essentially eliminating heat losses. All cells, whether conventional or ohmic, were designed to be mounted with their ends facing the test chamber electrodes (i.e., parallel to the current path). Fig. 1 shows a typical arrangement of the capillary cells and cell holder inside the ohmic treatment chamber.

Ohmic cells were equipped at each end with electrodes constructed of the same material as the sample, but in the form of alginate gels to prevent sample leakage. To ensure that the sample would allow the passage of current, the alginate electrodes were made more conductive than the sample and the test chamber fluid. We subsequently verified that the sample was indeed being ohmically heated by observing that the temperature of the ohmic cell was 1–3 °C higher than the outside solution (details later). This temperature gradient in favor of the ohmic cell ensured that any heat flow was entirely from the sample to the test chamber fluid, and confirmed that the sample was heated by pure ohmic heating.

Conventional cells were constructed similar to ohmic cells, but with non-conductive end pieces (clogs). As the clogs were not conductive to electricity, the test samples were heated by the flow of heat from the surrounding solution. Temperature distribution studies were conducted to ensure that the samples were heated conventionally. As the ohmic cells stayed at a slightly higher temperature than the test chamber fluid and conventional cells, separate experiments had to be conducted for conventional and ohmic treatments to match the same time–temperature histories in each case. In other words, conventional heating experiments were conducted with test chamber fluid at a marginally higher temperature than the ohmic experiments, so that sample temperature histories were the same in each case. Specially designed thermocouple cells, prepared similar to either ohmic or conventional cells, were used to precisely monitor the temperature inside the samples.

An elongated sleeve at the lower end of the treatment chamber was used as a cooling zone. Treated cells were transferred from the heating zone at the end of their respective holding times to the cooling section to rapidly cool the samples below 20 °C.