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Analysis of reduction of *Geobacillus stearothermophilus* spores treated with high hydrostatic pressure and mild heat in milk buffer

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Abstract

Our unpublished experimental results of fractional factorial experiments showed that the significant external factors affecting high pressure processing (HPP) inactivation were pressure, temperature and pressure holding time. Based on these results, response surface methodology (RSM) was employed in the present work and a quadratic equation for HPP inactivation was built. By analyzing the response surface plots and their corresponding contour plots as well as solving the quadratic equation, the experimental values were shown to be significantly in good agreement with predicted values since the adjusted determination coefficient (R^2_{Adj}) was 0.9747. The optimum process parameters for six log-cycles reduction of *Geobacillus stearothermophilus* spores were obtained as: temperature, 86 °C; pressure, 625.0 MPa and pressure holding time, 14.0 min. The adequacy of the model equation for predicting the optimum response values was verified effectively by the validation data.

Keywords: High pressure processing (HPP); Reduction of *Geobacillus stearothermophilus* spores; Response surface methodology (RSM); Effect; Mild heat

1. Introduction

Nowadays, consumer demands are directed more and more towards high-quality, minimally processed, nutritious and fresh-like products. Traditional thermal processing methods cause loss of desirable properties related to texture, flavor, color, and nutrient value. Food scientists and the food industry are therefore searching for novel methods that may destroy undesired micro-organisms with less adverse effects on product quality. Thanks to technological progress in the engineering aspects, however, physical alternative such as high pressure processing (HPP) is becoming

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more attractive. HPP offers an alternative potential non-thermal preservation method for pasteurization of food products. The major benefit of pressure is its immediate and uniform effect throughout different media, avoiding difficulties like non-stationary conditions typical for convection-type and conduction-type processes (Crawford et al., 1996; Knorr, 1993; O'Brien and Marshall, 1995; Patterson et al., 1995; Raso et al., 1998; Yuste et al., 1998; Matser et al., 2004). HPP treatment causes less deterioration of essential vitamins, phytochemicals, aroma compounds compared with the classical heat treatment, but microorganisms can be inactivated if the applied pressures are high enough (Hite, 1899; Cheftel, 1995). Microorganisms are variable with regard to their sensitivity to HPP (Hoover, 1993). Typically, 10-min exposures to HPP of 250–300 MPa at low or moderate temperature (<50 °C) result in what could be called 'cold pasteurization' (Hoover, 1997); however, in many cases bacterial endospores are not significantly inactivated by such treatments. Inactivation of dormant bacterial spores is the main objective of food sterilization. First attempts to affect bacterial spores with high-pressure application date back to the beginning of the 20th century (Larson et al., 1918; Ananta et al., 2001; Reddy et al., 1999). Their results showed that bacterial spores are more resistant than vegetative bacteria and may survive pressures above 1200 MPa. A combination of HPP with mild heat can be an effective method of spore inactivation (Sale et al., 1970; Seyderhelm and Knorr, 1992).

Despite the fact that a range of HPP-treated products has been on the Japanese market for several years, including fruit preparations, fruit juices, rice cakes and raw squid (Watanable et al., 1991), as yet, a perfect accredited inactivation model of HPP has not been established theoretically. Although there are many reports showing inactivation of bacterial spores, this issue has not yet been fully solved. The spore of *Geobacillus stearothermophilus* is the most heat-resistant species among aerobic spore-forming bacteria. This microorganism is often used as a biological indicator to evaluate sterilization processes because of its high heat resistance (López et al., 1997; Periago et al., 1998).

The objectives of this research were, therefore, to investigate the effects of high hydrostatic pressure and mild heat on *G. stearothermophilus* spores, one of the pressure and heat resistant Bacilli (Lechowich, 1993; Butz et al., 1990; Meyer et al., 2000), and to develop a response surface model using Central Composite design (Murthy et al., 2000; Ambati and Ayyanna, 2001) for predicting optimized processing conditions to inactivate *G. stearothermophilus* spores in milk buffer. The development of response surface model to describe the HPP inactivation of *G. stearothermophilus* spores should be very beneficial to applications in food preservation by optimizing process conditions and help construct a HACCP program to maintain food safety.

2. Materials and methods

2.1. Spore production and culture growth

A fresh, overnight culture of *G. stearothermophilus* (As 1.1923; China General Microbiological Culture Collection Center, China) was spread-plated on nutrient agar (Oxoid CM3, Basingstoke, UK) for 20 h at 55 °C. The purity of the obtained cultures was evaluated microscopically. After 1 week at room temperature, the percentage of sporulated cells was checked by differential phase contrast microscope (Optiphot, Nikon, Japan). When 99% of the cells were sporulated, the spores were harvested in a sterile bottle by flooding the surface of the culture with sterile physiological solution (0.85% NaCl solution) and by scrapping the plate with a bent glass rod. All the spore-suspensions were pasteurized at 80 °C during 30 min to kill all the vegetative forms. The suspensions were centrifuged and washed twice with physiological solution. Finally, the pellet was re-suspended in the sterile distilled water, placed in cryogenic vials (Nalgene, Rochester, NY) and subsequently frozen at −20 °C. The spore concentration remained constant at approximately 10^9 CFU ml^{-1} throughout the storage period.

2.2. HPP treatment

Prior to high-pressure treatment, the above described frozen spores of *G. stearothermophilus* were thawed. The spores were harvested by centrifugation at 3000 × *g* for 15 min, and resuspended in milk buffer to give approximately 1×10^9 CFU ml^{-1}. The milk buffer (Gutierrez et al., 2002) was chosen to contain the same amounts of minerals and lactose as whey from rennet casein; the buffer contained the follow-