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The effects of growth temperature and growth phase on the inactivation of *Listeria monocytogenes* in whole milk subject to high pressure processing

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Abstract

The aim of this study was to explore the effect of a wide range of growth temperatures, growth phases and plating media on the inactivation of *Listeria monocytogenes* by high pressure processing (HPP). In part one, *L. monocytogenes* was grown to mid-stationary phase at 4, 15, 25, 35 or 43 °C, inoculated into whole UHT milk at ~10^7 CFU/ml and high pressure processed at 400 MPa at room temperature (20–25 °C). Afterward, the HPP milk was plated on Tryptic Soy Yeast Extract Agar (TSYEA) and Modified Oxford Agar (MOX) to determine the degree of injury. For part two, cells were grown to mid-exponential, late-exponential or mid-stationary phase at 15 or 43 °C and processed in the same way. Time to reach a 5-log reduction was determined and data were analysed by ANOVA. The results from part one showed that both growth temperature and plating medium had a significant effect ($P<0.001$) on the inactivation of stationary phase *L. monocytogenes* by HPP. Tukey's pairwise comparisons revealed that the effects of all temperatures, except 35 and 43 °C, were significantly different ($P<0.05$). Cells grown at 15 °C were most sensitive to HPP, followed by cells grown at 4, 25 or 35 °C, with cells grown at 43 °C appearing to be the most resistant. Inactivation of cells grown at 4, 15 or 25 °C followed first order kinetics, whereas cells grown at 35 or 43 °C displayed non-linear inactivation kinetics due to tailing. In part two, both growth phase and plating medium had significant effects on the inactivation ($P\leq0.001$) of *L. monocytogenes* by HPP. Cells grown at 15 °C to mid-stationary phase were the most pressure-resistant when tested on both media, and were significantly more resistant ($P<0.05$) than cells grown at the same temperature to the other two phases of growth. There was no significant difference between mid- and late-exponential phase cells grown at 15 °C. When cells were grown at 43 °C, mid-exponential phase cells were significantly more sensitive ($P<0.05$) than either late-exponential or mid-stationary phase cells, with no difference between late-exponential or mid-stationary phase cells. It was postulated that membrane composition, stationary phase proteins and/or stress proteins may affect pressure resistance.

Keywords: High pressure processing (HPP); *Listeria monocytogenes*; Whole milk; Growth temperature; Growth phase; Injury; Inactivation kinetics

1. Introduction

High pressure processing (HPP) is a non-thermal food preservation method that utilises hydrostatic pressures in the range of 300–700 MPa (Stewart and Cole, 2001). Effects of HPP on foods include the inactivation of microorganisms, protein denaturation, enzyme activation or inactivation, and retention of quality and freshness (Knorr, 1993). HPP causes disruption of hydrophobic and ionic bonds but not covalent bonds, so many small molecules in foods, including flavour compounds and vitamins, are left intact (Farr, 1990). Foods processed by high pressure may then be of superior quality as compared with their thermally processed counterparts. HPP can therefore be used to meet current consumer demands for 'fresh', preservative free and minimally processed foods. HPP-treated salsas, guacamole, oysters and deli meats are available in the U.S. Despite numerous outbreaks of foodborne illness associated with the consumption of raw milk and raw milk cheeses (Headrick et al., 1998; Mazurek et al., 2004), many consumers believe that raw milk has superior taste and is healthier than pasteurised milk. In recent years consumer consumption of raw milk and raw milk products has increased (Gish, 2004). Therefore, a potential application of HPP could be for the elimination of pathogens from raw milk and so allow for the production of safe non-thermally pasteurised soft cheeses.

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Listeria monocytogenes is a ubiquitous foodborne pathogen which causes the rare, but potentially fatal, disease listeriosis. The elderly, pregnant women and immunocompromised individuals are at a much higher risk of contracting listeriosis than the general community (Farber and Peterkin, 1991). Listeriosis is most commonly associated with consumption of raw milk, soft cheeses, hot dogs and deli meats (Sutherland and Porritt, 1997). *L. monocytogenes* is Gram positive, rod shaped, facultatively anaerobic, psychrotrophic and relatively resistant to salt, drying and low pH (Seelinger and Jones, 1986). Due to its ability to grow at refrigeration temperatures in many ready-to-eat (RTE) foods, it is extremely important that *L. monocytogenes* be eliminated from these foods.

A variety of factors can affect the sensitivity of microorganisms to HPP, including the species and strain of the microorganism (Alpas et al., 1999; Benito et al., 1999; Robey et al., 2001), the growth temperature and growth phase prior to HPP (McClements et al., 2001; Casadei et al., 2002; Bull et al., 2005), the composition of the suspending medium (Patterson et al., 1995; Simpson and Gilmour, 1997), the presence of antimicrobial compounds in the suspending medium (Patterson and Kilpatrick, 1998; Alpas et al., 2000; Chen and Hoover, 2003), and the magnitude and duration of the applied pressure (Simpson and Gilmour, 1997; Alpas et al., 1998; Lucore et al., 2000; Ritz et al., 2000; Jordan et al., 2001). The effects of pressure on microorganisms in foods are also influenced by food components such as fat (Garcia-Graells et al., 1999). Very little has been reported on the effect of prior growth conditions on the pressure resistance of microorganisms. It has been shown that growth temperature affects the pressure sensitivity of *L. monocytogenes*, *Bacillus cereus* and *Pseudomonas fluorescens* in milk (McClements et al., 2001) and *Escherichia coli* in broth (Casadei et al., 2002). McClements et al. (2001) reported that exponential and stationary phase cells of *L. monocytogenes* grown at 30 °C were more resistant to HPP at 400 MPa than cells grown at 8 °C. Bull et al. (2005) recently reported that stationary phase cells of *L. monocytogenes* grown at 43 °C were more resistant to HPP (400 MPa) than cells grown at 15 °C.

L. monocytogenes can be injured during high pressure processing, which is usually detected by growth of cells on non-selective, but not on selective media (McClements et al., 2001). It is important to detect HPP-injured cells, as they can recover rapidly in milk during storage (Hayman, 2001; Bozoglu et al., 2004; Bull et al., 2005). McClements et al. (2001) reported that counts of *L. monocytogenes* on a non-selective agar were higher than on a selective agar following HPP treatment of UHT milk.

The objective of this study was to explore the effect of a wider range of growth temperatures and growth phases on inactivation of *L. monocytogenes* subject to HPP as determined on both a selective and a non-selective medium. In addition, enumeration rather than enrichment was used to determine the inactivation kinetics of *L. monocytogenes* in UHT whole milk by high pressure processing.

2. Materials and methods

A strain of *L. monocytogenes* serotype 4b (ATCC 19115) was maintained in glycerol stock at −80 °C. All media were obtained from Difco (Becton, Dickinson and Company, Sparks, MD, USA) unless otherwise stated. A loopful of glycerol stock was streaked onto Tryptic Soy Agar supplemented with 0.6% yeast extract (TSYEA). The plate was incubated at 35 °C overnight, and then one colony was inoculated into 10 ml of Tryptic Soy Broth supplemented with 0.6% Yeast Extract (TSYEB) which was then incubated at 35 °C for 18–24 h. The resulting culture was diluted to ~10^7 CFU/ml in 0.1% peptone water, and 1 ml of diluted culture was added to 100 ml of TSYEB.

For part one broths were incubated at 4, 15, 25, 35 or 43 °C, for 2 weeks, 50 h, 24–26 h, 14–16 h or 14 h respectively, to obtain mid-stationary phase cells. Growth curves at each growth temperature were determined in advance by both plating and measurement of absorbance at 625 nm (data not shown). Growth phase was subsequently confirmed by absorbance at 625 nm in all experiments. One ml of stationary phase culture was added to 100 ml Paramlat UHT whole milk at 4 °C to obtain an initial inoculum of ~10^7 CFU/ml. Tips were aseptically cut off 3.1 ml plastic transfer pipettes (Sigma-Aldrich, Milwaukee, WI, USA) and 5 ml of inoculated milk was aseptically added to each plastic pipette bulb using a sterile syringe. Pipette bulbs were heat sealed by melting in a Bunsen flame and pressing with a heat sealer (MP-12, Impulse; Midwest Pacific, St. Louis, MO, USA). Sealed pipette bulbs were placed in 4 in.×6 in. stomacher bags (VWR, West Chester, PA, USA) containing 1%

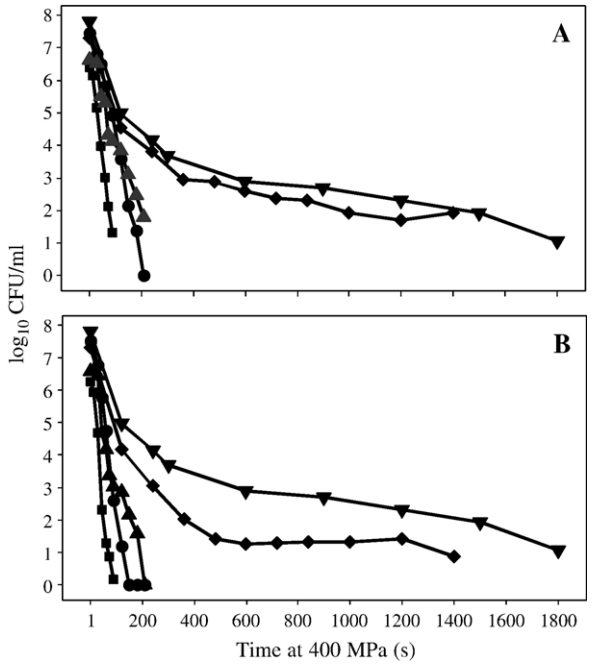

Fig. 1. Effect of growth temperature and plating medium on the numbers of *Listeria monocytogenes* recovered after high pressure processing (HPP) at 400 MPa. *L. monocytogenes* was grown to mid-stationary phase in Tryptic Soy Yeast Extract Broth at 4 °C (●), 15 °C (■), 25 °C (▲), 35 °C (◆) or 43 °C (▼). Whole UHT milk was inoculated with *L. monocytogenes* to ~10^7 CFU/ml and HPP at 400 MPa was applied at ambient temperature (20–25 °C) for various times. After HPP milk samples were spread on plates of Tryptic Soy Yeast Extract Agar (A) or Modified Oxford Agar (B) which were incubated at 35 °C for 48 h.