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Effect of high-pressure processing on reduction of *Listeria monocytogenes* in packaged Queso Fresco[1]

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ABSTRACT

The effect of high-hydrostatic-pressure processing (HPP) on the survival of a 5-strain rifampicin-resistant cocktail of *Listeria monocytogenes* in Queso Fresco (QF) was evaluated as a postpackaging intervention. Queso Fresco was made using pasteurized, homogenized milk, and was starter-free and not pressed. In phase 1, QF slices (12.7 × 7.6 × 1 cm), weighing from 52 to 66 g, were surface inoculated with *L. monocytogenes* (ca. 5.0 $\log_{10}$ cfu/g) and individually double vacuum packaged. The slices were then warmed to either 20 or 40°C and HPP treated at 200, 400, and 600 MPa for hold times of 5, 10, 15, or 20 min. Treatment at 600 MPa was most effective in reducing *L. monocytogenes* to below the detection level of 0.91 $\log_{10}$ cfu/g at all hold times and temperatures. High-hydrostatic-pressure processing at 40°C, 400 MPa, and hold time ≥15 min was effective but resulted in wheying-off and textural changes. In phase 2, *L. monocytogenes* was inoculated either on the slices (ca. 5.0 $\log_{10}$ cfu/g; ON) or in the curds (ca. 7.0 $\log_{10}$ cfu/g; IN) before the cheese block was formed and sliced. The slices were treated at 20°C and 600 MPa at hold times of 3, 10, and 20 min, and then stored at 4 and 10°C for 60 d. For both treatments, *L. monocytogenes* became less resistant to pressure as hold time increased, with greater percentages of injured cells at 3 and 10 min than at 20 min, at which the lethality of the process increased. For the IN treatment, with hold times of 3 and 10 min, growth of *L. monocytogenes* increased the first week of storage, but was delayed for 1 wk, with a hold time of 20 min. Longer lag times in growth of *L. monocytogenes* during storage at 4°C were observed for the ON treatment at hold times of 10 and 20 min, indicating that the IN treatment may have provided a more protective environment with less injury to the cells than the ON treatment. Similarly, HPP treatment for 10 min followed by storage at 4°C was the best method for suppressing the growth of the endogenous microflora with bacterial counts remaining below the level of detection for 2 out of the 3 QF samples for up to 84 d. Lag times in growth were not observed during storage of QF at 10°C. Although HPP reduced *L. monocytogenes* immediately after processing, a second preservation technique is necessary to control growth of *L. monocytogenes* during cold storage. However, the results also showed that HPP would be effective for slowing the growth of microorganisms that can shorten the shelf life of QF.

Key words: high-pressure processing, Queso Fresco, *Listeria monocytogenes*, microbial inactivation

INTRODUCTION

In the United States, fresh cheeses are manufactured from pasteurized milk to eliminate pathogens, if present, and to lower the levels of microflora that can reduce shelf life and affect the textural and sensory properties of the cheese. One of the most popular of the fresh cheeses is Queso Fresco (**QF**), which is a Hispanic-style cheese distinguished by its bright white texture, crumbliness, mild salty flavor and non-melting characteristics. However, its high pH and moisture content provide the ideal conditions for growth of bacteria and other microflora, which can limit its shelf life (Leggett et al., 2012). Despite the use of pasteurized milk, QF and other fresh cheeses made commercially have been subject to occasional recalls, most likely due to environmental contamination by *Listeria monocytogenes* (Lin et al., 2006; Soni et al., 2010).

Postpasteurization *L. monocytogenes* contamination likely occurs both at the surface and the interior of

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the cheese due to processing steps that may involve handling of the curd and use of utensils (Sandra et al., 2004; Soni et al., 2010; Leggett et al., 2012) or during the milling step in manufacture, which is used to impart a crumbly texture to the curd (Van Hekken et al., 2012). Although larger, modern QF plants conduct manufacturing in closed vats so that the cheese has little contact with the environment, the hoops used for pressing and shaping the cheese may be packed by hand.

In a study to assess the viability of *L. monocytogenes* when introduced as an environmental pathogen, Leggett et al. (2012) demonstrated that QF inoculated with *L. monocytogenes* showed a maximum population density of 7.80 $\log_{10}$ cfu/g of cheese after 20 d of storage at either 4 or 10°C. The levels of indigenous bacteria and other microflora, such as yeasts and molds, also increased significantly by 28 d of storage. The use of postlethality interventions such as high-hydrostatic-pressure processing (**HPP**; Hnosko et al., 2012; Leggett et al., 2012) on packaged cheese or the inclusion of antimicrobials (Soni et al., 2010, 2012) in the packaging of the cheese have been suggested to control the growth of *L. monocytogenes* during cold storage and additionally slow or prevent the growth of indigenous bacteria, thus extending shelf life.

The application of pressure to a sample through a pressurized fluid via HPP is an effective method for inactivation of pathogens, such as *L. monocytogenes* and spoilage organisms under room temperature conditions, and spores at elevated temperatures, in a variety of food products such as guacamole, salsa, fruit juices, meats, and seafood (Rastogi et al., 2007; Zhang and Mittal, 2008; Simonin et al., 2012). High-hydrostatic-pressure processing is growing as a processing method or intervention technology of choice because of its demonstrated ability to economically extend shelf life and preserve the quality of food, as heat is not applied. Unlike interventions, such as antimicrobials or oils, which are usually applied to the surface of a food product, HPP operates according to the isostatic principle, in which the pressure applied to a sample through a pressurized medium such as water or oil is instantaneous and uniform throughout the sample, regardless of its volume or shape, thus inactivating microbes throughout a sample (Rastogi et al., 2007).

Although HPP is typically conducted under room temperature conditions, the temperature of the solid or liquid sample and the pressurizing fluid will increase when pressure is applied, assuming no loss of heat from the walls of the pressure chamber. This is due to the work of adiabatic compression, which interrupts the intermolecular forces of the pressuring fluid and the sample, causing a temperature increase in both (Denys et al., 2000; Ardia et al., 2004; Knoerzer and Versteeg, 2009). The compression heating factor ($\boldsymbol{\Delta T/\Delta P}$) is given by

$$\Delta T/\Delta P = \beta T/\rho C_p, \quad [1]$$

where T is the temperature (absolute K), P is the pressure (Pa), β is the coefficient of thermal expansion (K^{-1}), ρ is the density (kg/m^3), and C_p is the heat capacity (J/kg·K).

In accordance with Le Chatelier's principle, interruption of intermolecular forces by pressurization leads not only to an increase in sample temperature, which in the case of cheese, depends on composition (Hnosko et al., 2012; Van Hekken et al., 2013), but a decrease in the volume of water, which is a response by the sample to restore the various equilibria that were in operation before HPP (Huppertz et al., 2006). For cheese, adjustments in the intermolecular forces are reflected in changes in the microbiological, physicochemical, rheological, and sensory properties upon HPP treatment and after (Martínez-Rodríguez et al., 2012). High-hydrostatic-pressure processing treatment of cheese has been shown to lead to a reorganization of water molecules around the ions, changes in the amount of free and unbound water molecules (Martínez-Rodríguez et al., 2012), and more compact structures, which affect the mineral balance of cheese, enzyme interactions, and protein conformation (Knorr et al., 2006). The covalent bonds remain intact after HPP but the secondary structures are denatured and changes in the tertiary structures, maintained by the hydrophobic and ionic interactions, occur at applied pressures >200 MPa (Hendrickx et al., 1998; Chawla et al., 2011).

High-hydrostatic-pressure processing applied to QF does not significantly change its traditional properties. For QF in particular, which is known for its color and crumbliness, HPP conducted at 400 MPa resulted in QF that was more yellow compared with the control (Sandra et al., 2004). However, Van Hekken et al. (2013) found that QF made without starter culture and not pressed, treated by HPP at 200 or 400 MPa at an initial cheese temperature of 20°C, had the same color as untreated samples. Only QF treated at an initial temperature of 40°C by HPP at 200, 400, or 600 MPa was slightly more yellow than the control. Crumbliness of QF was not adversely affected with treatment at 400 MPa, a 20-min hold time, and 20°C compared with the control (Sandra et al., 2004). Van Hekken et al. (2013) also found that HPP at 200, 400, and 600 MPa and initial QF temperature of 20°C with up to a 20-min hold time resulted in a texture similar to the control. However, for QF manufactured with a starter culture and pH 5.0, HPP applied at 400 and 600 MPa for 1