Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Inactivation of *Staphylococcus aureus* by high hydrostatic pressure in saline solution and meat slurry with different initial inoculum levels

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ABSTRACT

The effect of high hydrostatic pressure (350–550 MPa, 3–12 min) and initial inoculum levels (10^2–10^6 CFU/mL (CFU/g)) on *Staphylococcus aureus* at room temperature in saline solution and meat slurry were investigated, and the Weibull model was used to describe the inactivation of *S. aureus* in both media. The results showed that the reduction of *S. aureus* was enhanced with increasing the pressure level and the pressure holding time in both media. The inactivation rate of *S. aureus* increased with increasing the initial inoculum level in saline solution, whereas 10^4 CFU/g of initial inoculum caused the higher log-reduction of *S. aureus* than the other two initial inoculum levels in meat slurry. Regression coefficients (R^2), root mean square (RMSE) values and residual plot suggested that the Weibull model produced a good fit to the data. However, the shape factor (n) was not significantly dependent on the pressure and initial inoculum level. Multiple linear regression was further used to describe the effect of pressure and log initial inoculum level on the scale factor (b) values. Based on the primary and secondary models, the inactivation of *S. aureus* in the both two media was predicted satisfactory by the tertiary model. In addition, 3 log-reduction of *S. aureus* was obtained by the combination treatment of HHP (350 MPa for 6 min) and nisin (500 ppm), which has met Food Safety Standard on Quick Frozen Flour and Rice Products for microorganism in the People's Republic of China.

Keywords: *Staphylococcus aureus*; Meat slurry; High hydrostatic pressure; Initial inoculum level; Weibull model; Nisin

1. Introduction

Staphylococcus aureus is a major causative organism responsible for food intoxications. It has been isolated from several foods, such as meat and meat products, milk and dairy products, and salads. In many countries, *S. aureus* is considered to be the second most common pathogen causing outbreaks of food poisoning after *Salmonella* (EFSA, 2007; Kérouanton et al., 2007). In 2011, the China Centers for Disease Control (CDC) reported that *S. aureus* was detected in the minced meat of quick-frozen dumplings and steamed stuffed buns (CDC, 2011). Because of a prevalence of *S. aureus* as high as 25.9% (detected by culture) or 51.1% (detected by PCR) in fresh pork (Atanassova et al., 2001) and the hygienic practices involved in handling minced meat during different steps of processing (cutting, grinding, and preparation), there is a certain risk of staphylococcal food poisoning from the consumption of minced meat products.

High hydrostatic pressure (HHP) is one of the methods proposed to control *S. aureus* in food. Many of the previous studies on HHP-induced *S. aureus* inactivation were conducted in milk and dairy products or meat products (Ananou et al., 2010; López-Pedemonte et al., 2007; Pérez Pulido et al., 2012; Tassou et al., 2007, 2008), and relatively little research has been

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Available online 8 July 2014
http://dx.doi.org/10.1016/j.fbp.2014.06.005

performed in fresh meat. However, fresh meat has the potential danger being contaminated with *S. aureus* (Atanassova et al., 2001). In order to guarantee the safety of fresh meat before being processed to other products, the use of HHP to control *S. aureus* contamination in fresh meat needs to be studied. The resistance of *S. aureus* to HHP is highly variable, mainly depending on its physiological state and the food matrix (Alpas et al., 2003). With respect to the food matrix, numerous studies have shown that a nutrient-rich medium has a more protective effect on microorganism against pressure than an aqueous buffer medium (Panagou et al., 2007; Hugas et al., 2002; Chen and Hoover, 2003; Kalchayanand et al., 1998). However, different results were also observed in some special cases. For instance, Garriga et al. (2002) observed no significant difference in the inactivation of *Escherichia coli* CTC1018 at 500 MPa for 10 min in cooked ham homogenized with water (3:1) and in phosphate buffer. Patterson et al. (1995) found that *Listeria monocytogenes* NCTC 11994 and *E. coli* O157:H7 NCTC 12079 were more resistant to pressure when treated in UHT milk than in poultry meat or buffer. These contradictory findings indicated that the inactivation of microorganisms in buffer is unlikely to represent real food systems.

To predict the bactericidal effect of HHP on *S. aureus* in meat slurry matrix, inactivation kinetics are essential to establish the safe processing conditions (Chen and Hoover, 2003). Recent studies showed that the microbial inactivation curve under high pressure can be described by a number of models, including Cerf, Weibull, modified Gompertz, and Log-logistic (Buzrul et al., 2005; Wang et al., 2013). Among them, the Weibull model is gaining wide acceptance because of its simplicity and flexibility. Recently, the Weibull model has been successfully used in describing the inactivation of different microorganisms by HHP (Chen and Hoover, 2004; Dilek Avsaroglu et al., 2006). In this study, we used the Weibull model to describe the inactivation curves of *S. aureus* exposed to HHP in saline solution and meat slurry. In addition, it is economically beneficial to use lower pressure in combination with other techniques to obtain the desired inactivation effect and product quality in food processing (Simonin et al., 2012). Nisin is a natural antimicrobial peptide produced by certain strains of *Lactococcus lactis* (De Vuyst, 1994). It is effective against a wide range of gram-positive bacteria (de Arauz et al., 2009). However, little information is available about the combined effect of HHP and nisin on the inactivation of *S. aureus*.

The objective of this work was to investigate the inactivation effect of *S. aureus* with different initial inoculum levels by HHP in saline solution and meat slurry, and model the inactivation kinetics of *S. aureus* in the both two media. It could help the meat industry further to develop optimum process conditions for the meat with different contamination degree of *S. aureus*. Furthermore, the combined effect of HHP and nisin on the inactivation of *S. aureus* was also assessed.

2. Materials and methods

2.1. Cultures and media

S. aureus (CGMCC 1.1861, ATCC 6538P), a food poisoning strain and widely used in media testing, sterility testing and susceptibility testing, was obtained from the China General Microbiological Culture Collection Center (CGMCC, Beijing, China), maintained on slants of Nutrient Agar (NA, Beijing Aoboxing Biological Technology Co., Ltd., Beijing, China) at 4 °C, and subcultured before using.

2.2. Preparation of inoculum

A loop of *S. aureus* was transferred from the nutrient agar slant to 100 mL of nutrient broth and inoculated at 37 °C for 16 h as the stock culture. For the preparation of the inoculum, *S. aureus* was prepared by inoculating nutrient broth (100 mL) with 1 mL of stock culture and incubating at 37 °C for18 h with shaking at 200 rpm. During the growth of *S. aureus*, early stationary cells were harvested by centrifugation at 7000 × *g* at 4 °C for 15 min and then washed twice with a 0.85% sterile saline solution and pelleted again. *S. aureus* suspensions had a population of ca. 7 log CFU/mL.

2.3. Inoculation of the saline solution and meat slurry

For the inoculation of the saline solution, the prepared inoculum was resuspended in saline solution to approximately 5×10^6 CFU/mL. Then, the bacterial suspension was diluted to 10^4 and 10^2 CFU/mL. All of the cell suspensions were vacuum-packaged in sterile polyethylene–polyamide plastic bags (20 mL per pouch), stored at 4 °C, and treated within 3 h.

For the inoculation of the meat slurry, fresh pork was minced at a ratio of 1 to 1 with fat and lean meat (including 37% fat, 13.2% protein, 46.8% water), and 10% (v/w) cell suspensions were inoculated into the meat slurry to obtain a population of approximately 5×10^6, 10^4 and 10^2 CFU/g. All of the samples were vacuum-packaged in polyethylene–polyamide plastic bags (50 g per pouch) and stored at 4 °C until pressurization.

2.4. HHP treatment of the inoculated saline solution and meat slurry

HHP treatments were applied to cells suspended in saline solution and inoculated into meat slurry in a hydrostatic pressurization unit (CAU-HHP-700-6, Baotou Kefa High Pressure Technology Co., Ltd., Baotou, China) with a capacity of 7 L and maximum pressure of 700 MPa. The pressure transmission fluid was distilled water. The rate of pressure increase was 133 MPa/min, and the pressure release was immediate. The come-up and pressure-release times were not included in the pressurization time. The inoculated saline solution and meat slurry were divided into three groups, respectively. The first group represented an unpressurized sample as a control, the second group represented the pressurization process (350, 450 and 550 MPa for 3, 6, 9, 12 and 15 min, respectively), and the third group (with initial bacterial inoculum of approx. 5×10^6) represented the pressurization process (350, 450 and 550 MPa for 6 min) with 500 ppm nisin (1×10^6 IU/g, Zhejiang Silver-Elephant Bio-engineering Co., Zhejiang, China) treatment. The 500 ppm was consistent with the maximal amount according to the standard code for food additives in the People's Republic of China (GB2760-2011). The initial temperature in the processing vessel is nearly 25 °C and when 550 MPa pressure is applied to the samples, the temperature reached 41.5 °C which is calculated as 3 °C/100 MPa (Balasubramaniam et al., 2008). When the pressurization was finished, the sample temperature quickly dropped to its initial temperature due to heat transfer from the samples to the stainless steel of the vessel, so its contribution to the destruction of microorganisms by HHP was considered negligible. Each experiment was repeated