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ORIGINAL ARTICLE

Temperature-assisted high hydrostatic pressure inactivation of *Staphylococcus aureus* in a ham model system: evaluation in selective and nonselective medium

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Abstract

Aims: The purpose of this study was to investigate the inactivation kinetics of *Staphylococcus aureus* in a ham model system by high hydrostatic pressure at ambient (25°C) and selected temperatures (45, 55°C). Selective [Baird Parker (BP) agar] and nonselective [brain heart infusion (BHI) agar] growth media were used for enumeration in order to count viable and sublethally injured cells.

Methods and Results: The micro-organism was exposed to a range of pressures (450, 500, 550, 600 MPa) at ambient temperature (25°C) for up to 45 min. Additionally, the behaviour of the micro-organism was evaluated at mild temperatures in combination with high pressure treatment, namely: (i) 350, 400 and 450 MPa at 45°C; and (ii) 350 and 400 MPa at 55°C, for up to 12 min. Inactivation kinetics were calculated in terms of D_p and z_p values. Survival curves of *S. aureus* at ambient temperature were mostly linear, whereas when temperature was applied, tailing was observed in most survival curves. The estimated D_p values and therefore the number of surviving cells, were substantially higher on the selective BP agar in the whole range of pressures applied, indicating that *S. aureus* showed greater recovery in the selective BP agar than the nonselective BHI agar. Samples pressurized at ambient temperature needed higher pressures (over 500 MPa) to achieve a reduction of the population of the pathogen more than 5 log CFU ml^{-1}. The same level of inactivation was achieved at lower pressure levels when mild heating was simultaneously applied. Indeed, more than 6 log CFU ml^{-1} reductions were obtained at 400 MPa and 55°C within the first 7 min of the process in BHI medium.

Conclusion: Elevated temperatures allowed lower pressure levels and shorter processing times of pathogen inactivation than at room temperature. Greater recovery of the pathogen was observed in the selective (BP agar) medium, regardless of pressure and temperature applied.

Significance and Impact of the Study: The obtained kinetics could be employed by the industry in selecting optimum pressure/temperature processing conditions. Attention must be given to the selection of the enumeration medium, as the use of an inappropriate medium would lead to underestimation of the surviving cells, thus imposing a risk in the microbiological safety of the product.

Introduction

In recent years, considerable research efforts have been focussed on the development of nonthermal processes for food preservation, such as the use of high hydrostatic pressure, pulsed electric field, ultraviolet light, ionizing radiation, pulsed light, and more recently, ultra high pressure homogenization (Sale *et al.* 1970; Zhang *et al.* 1995; Qin *et al.* 1996; Gervilla *et al.* 2000; Diels *et al.* 2004). High hydrostatic pressure has been proposed as an emerging nonthermal food preservation technique to eliminate foodborne pathogens and consequently enhance the safety and shelf life of foods without changing the sensorial attributes of the product (Hoover 1993; Cheftel 1995; Smelt 1998; Tewari *et al.* 1999; Patterson 2005; Torres and Velazquez 2005). Research into the application of high-pressure processing started more than a century ago when Hite *et al.* provided evidence that the shelf life of milk, fruit and vegetables could be extended by high pressure treatment (Hite 1899; Hite *et al.* 1914); however, it is only in the last 25 years that it has become a commercial reality. Today, a variety of pressure-treated products, such as jams, fruit juices, avocado salad (guacamole), fresh-cut fruit salads, fresh whole oysters, etc. are commercially available in the United States, Europe and Japan (Torres and Velazquez 2005), whereas another potential application in the food industry is the production of a range of novel meat, poultry, fish, and dairy products. However, as foods are frequently implicated as vehicles of foodborne pathogens, it is important to provide information on the effect of high-pressure processing on these micro-organisms. The exact mechanism by which high-pressure inactivation of micro-organisms takes place has not been fully elucidated, but it is generally believed that the application of high pressures causes morphological, biochemical and genetic alterations resulting in cell death owing to multiple or accumulated damage (Hoover *et al.* 1989; Cheftel 1995; Simpson and Gilmour 1997). Studies on pathogens showed that cell inactivation by high pressure is strongly related to the cell wall type and cellular morphology (Cheftel 1995; Ludwig and Schreck 1997; Kalchayanand *et al.* 1998). Consequently, yeasts and fungi are very sensitive to high pressure and Gram-positive micro-organisms are more resistant, possibly owing to their cell wall structure, and finally Gram-negative micro-organisms are more sensitive. In addition, cocci-shaped cells are more resistant than rod-shaped cells.

High-pressure processing, as a mild treatment of food, has good potential for the meat industry. Sliced vacuum-packed meat products are highly perishable owing to their composition, pH and water activity, in addition to the lack of an indigenous microflora competing with spoilage and pathogenic micro-organisms. Their shelf life depends on the hygienic characteristics of the final product after postprocessing and the packaging methods, where post-contamination is more likely to occur (Hugas *et al.* 2002). High-pressure processing can successfully find application in sliced cooked ham and other delicatessen meat products sold in flexible pouches. A significant delay in the growth of spoilage micro-organisms can be attributed to high pressure application, thus minimizing the possibility for off-flavour and gas production (Garriga *et al.* 2004; Morales *et al.* 2006).

One of the most high-pressure-resistant nonsporulated Gram-positive bacteria is *Staphylococcus aureus* (Patterson *et al.* 1995; Alpas *et al.* 1999; Wuytack *et al.* 2002). The most significant virulence factor related to *S. aureus* is the production of heat-stable enterotoxins, the consumption of which by susceptible individuals may produce symptoms, such as nausea, vomiting, diarrhoea, abdominal cramps and gastroenteritis (Dinges *et al.* 2000; Kérouanton *et al.* 2007). Staphylococcal enterotoxins are proteins produced by about 25% of *S. aureus* strains isolated from foods (Dinges *et al.* 2000; Belay and Rasooly 2002; Cenci-Goga *et al.* 2003). In France, *S. aureus* represents the second most common cause of foodborne disease after *Salmonella* (Kérouanton *et al.* 2007), whereas in the United States, Staphylococcal enterotoxin A is the most frequently encountered micro-organism from food-poisoning outbreaks (Balaban and Rasooly 2000). High-pressure inactivation of *S. aureus* in buffer systems and in a diversity of food commodities has been extensively studied (Takahashi 1992; Patterson and Kilpatrick 1998; Gervilla *et al.* 1999; O'Reilly *et al.* 2000; Alpas *et al.* 2003; Diels *et al.* 2003; Gao *et al.* 2006; Briñez *et al.* 2007; Buzrul and Alpas 2007). However, in most of these studies, pressure resistance has not been investigated at different pressures as a function of time which would result in the determination of kinetic data (e.g. decimal reduction times, thermal death times) that can be employed directly by the industry. Instead, the pathogen is subjected in a predefined set of pressure/time, or a series of pressures for the same time, and the reduction in the initial population is reported.

Investigating the resistance of pathogens is important in recovering stressed cells, which, under certain circumstances, can grow and cause problem. It is known that selective growth media allow for differentiation and enumeration of specific target micro-organisms, but these media also contain agents which may inhibit repair of sublethally injured cells (Wuytack *et al.* 2002; Yuste *et al.* 2004). Sublethally injured cells are therefore not detected on selective media, thus increasing the risk of overestimating the efficacy of the pressure treatment.

The aim of this work was: (i) to investigate the effect of temperature-assisted high-pressure inactivation kinetics

of a barotolerant strain of *S. aureus* in a ham model system; and (ii) to compare the sensitivity of selective and nonselective solid media in the enumeration of viable and sublethally injured cells of the pathogen.

Materials and methods

Bacterium and preparation of cell suspension

A strain of *S. aureus*, isolated previously from vacuum-packaged sliced ham after high-pressure treatment of 450 MPa for 5 min, was used as a test micro-organism. A characterization of the isolated strain, including antibiotic resistance and growth characteristics, has been reported recently (Karatzas *et al.* 2007). Stock cultures for the assays were maintained in vials of treated beads (Protect bacterial preservers; Technical Service, Lancashire, UK) at −80°C until use. Prior to experimentation, one cryobead was inoculated into 100 ml brain heart infusion broth (BHI; Merck 1.10493, Darmstadt, Germany) and incubated at 37°C for 24 h. The culture was kept at 4°C and renewed periodically to ensure viability. For growth, a loopful of the culture was transferred in 100 ml BHI broth and incubated at 37°C for 18–20 h. The cells were harvested by centrifugation (7000 ***g*** for 10 min at 4°C), washed twice with sterile phosphate-buffered solution (pH 6·8), re-centrifuged and finally suspended in the ham model system to give a final concentration of about 10^8 cells per ml as assessed by the microscopic count with a Neubauer counting chamber (Brand, Wertheim, Germany). The ham model system was a well-homogenized mixture of ham and water (4·4 : 1 or 52·36 g ham + 12 ml H_2O).

High-pressure equipment

The high-pressure system (Resato International B.V., Roden, Holland) consists of a high-pressure intensifier unit for the build-up of pressure in the system and an electric motor to drive a hydraulic pump. The oil in the pump is used to propel the oil-driven double-acting intensifier, which is actually a hydraulically driven reciprocating pump. In the intensifier, the pressure of the high-pressure fluid (Resato International B.V. high-pressure fluid-glycol emulsion; Resato International B.V., Roden, Holland) is ranged up to 1000 MPa. The pressure is adjustable in steps of approximately 25 MPa.

Moreover, the system consists of a block of six small (42 ml) high-pressure vessels measuring 2·5 cm in diameter and 10 cm in length, respectively (Fig. 1). The vessels are closed with a unique Resato thread connection on the top of the vessel. The pressure is transmitted from the intensifier to the vessels by the pressure fluid through high-pressure stainless steel tubing. Air-operated high-pressure needle valves are used for the control of circulation of pressure fluid, so that each vessel operates independently. Each vessel is equipped with a heating/cooling jacket to control experimental temperature in a range from -40 to +100°C. Temperature transmitters are mounted in each vessel to monitor temperature. Finally, two pressure transducers are used to monitor the pressure in the system.

Figure 1 Block of six small (42 ml) high-pressure vessels (2·5 cm in diameter and 10 cm in length) used for the pressurization of the ham model system pouches.

Pressurization of samples

Aliquots of 3·0 ml of sample were transferred in polyethylene pouches (20 mm width × 80 mm length) and heat-sealed taking care to expel most of the air. Every pouch was placed in a second slightly bigger one to prevent accidental leakage of cell suspension and contamination of the pressurizing liquid. The pouches were placed in duplicate in each of the six small vessels of the high-pressure unit. The vessels were filled up with pressurizing liquid and their caps were screwed tightly. The initial temperature of the vessel jacket was adjusted to 25°C by means of a water–glycerol solution circulating from a water bath. In order to study the kinetics of cell inactivation under the same experimental conditions with only one variable (hold time at pressure), the desired pressure was applied and at predetermined time intervals, different for each assay, the pressure was released from each vessel; hence, a data set for each time/pressure combination was obtained.

Initially, samples were subjected to 450, 500, 550 and 600 MPa at 25°C (ambient temperature). Moreover, in order to determine the effect of treatment temperature on the survival of *S. aureus*, the samples were exposed to 350, 400 and 450 MPa at 45°C, in addition to 350 and

400 MPa at 55°C, respectively. The pressure in the system and the temperature in each vessel was monitored and recorded through the PLC (Pressure Level Control) system (Resato International B.V.). The come-up rate was approximately 100 MPa per 7 s and the pressure release time was less than 3 s. Pressurization time reported in this work did not include the pressure come-up and release times. Overall, the experiment was repeated twice with duplicate pouches for each combination of pressure, temperature and time.

Enumeration of survivors

At the onset of the experiment (i.e. before subjecting pouches to high pressure) and after treatment, pressurized pouches were removed from the vessels and their contents were aseptically diluted in ¼ sterile Ringer's solution (Merck 1.15525, Darmstadt, Germany). One hundred microlitres (100 μl) of at least three serial dilutions were spread-plated in triplicate on the nonselective BHI agar (BHI; Merck 1.13825, Darmstadt, Germany) and the selective Baird Parker agar (BP; Merck 1.05406, Darmstadt, Germany) for the growth of *S. aureus*. The plates were initially incubated at 37°C for 24 h to form visible colonies, and then re-incubated at the same temperature for an additional 48 h to allow injured cells to recover. The data from the plate counts were transformed to $\log_{10}$ values prior to further analysis.

Curve fitting

The survival curves of *S. aureus* during high-pressure inactivation were fitted with the model of Geeraerd *et al.* (2005). The general form of the model is the following:

$$N(t) = (N(0) - N_{res}) \cdot e^{-k_{\max} t} \cdot \left(\frac{e^{k_{\max} S_l}}{1 + (e^{k_{\max} S_l} - 1) \cdot e^{-k_{\max} t}} \right) + N_{res} \quad (1)$$

where, $N(t)$ (CFU ml^{-1}) represents the microbial cell density at time t, $N(0)$ (CFU ml^{-1}) the initial microbial cell density, N_{res} (CFU ml^{-1}) is the residual population density, $k_{\max}$ (1/time unit) is the specific inactivation rate, and S_l (time units) a parameter representing the shoulder. In this form, all parameters have a clear biological/graphical meaning and the three phases of inactivation (shoulder, log-linear phase and tailing region) are included in the model. In our experiments, no shoulder was observed in any of the applied pressures; hence, for $S_l = 0$, eqn (1) becomes:

$$N(t) = (N(0) - N_{res}) \cdot e^{-k_{\max} t} + N_{res} \quad (2)$$

It should be noted that when applying eqn (2) to experimental data, where the $\log_{10}$ transformation has been employed (as it is common in food microbiology), the following format is used:

$$\log_{10} N(t) = \log_{10} \left((10^{\log_{10}(N(0))} - 10^{\log_{10}(N_{res})}) \cdot e^{-k_{\max} t} + 10^{\log_{10}(N_{res})} \right) \quad (3)$$

The pressure survivor curves were plotted as $\log_{10} N/N_0$, where N_0 is the initial population of the pathogen and N is the surviving population at time t. Nonlinear regression was carried out using the Quasi-Newton algorithm of the NLIN procedure of Statistica release 6.0 (Statsoft Inc., Tulsa, OK, USA) to fit the ratio $log_{10}N_t/N_0$ for the determination of the specific inactivation rate ($k_{\max}$). The decimal reduction time (D_p) values for each treatment could then be estimated as $ln(10)/k_{\max}$. From the pressure death time (PDT) curve, the z_p value (i.e. the change in pressure required to accomplish a 10-fold increase or decrease in the D value) can be estimated as the negative reciprocal of the slope. The goodness-of-fit of survivor curves was assessed using the coefficient of determination (R^2) and the root mean squared error (RMSE). In addition, a t-test was performed at each pressure/temperature combination to compare the D_p values between the two enumeration media at $P < 0{\cdot}05$.

Results

The survival curves of *S. aureus* subjected to pressures from 450–600 MPa at 25°C, as enumerated in the selective (BP agar) and nonselective (BHI agar) media, are shown in Figs 2 and 3, respectively. Higher pressures were found to be more effective in the inactivation of microbial cells as shown both graphically by the increased steepness of the curves and numerically by the higher values of the inactivation rates ($k_{\max}$) and the corresponding lower D_p values (Table 1). Generally, the number of survivors was higher in the selective medium (BP agar) compared with the nonselective BHI agar. For example, a treatment of 600 MPa for 7 min reduced the population of *S. aureus* by 5·4 log CFU ml^{-1} when enumerated in the nonselective medium, whereas for the selective BP agar, such reduction was not achieved throughout the 18 min duration of the treatment at the same pressure level. The difference in the recovery of the micro-organism was very well depicted in the z_p values between the two enumeration media (Table 1 and Fig. 4), where it can be concluded that *S. aureus* was twice as resistant when the selective BP agar was used (z_p = 303 MPa) compared with the nonselective BHI (z_p = 154 MPa).

Simultaneous application of high pressure and elevated temperatures resulted in a downward curvature of the survival curves in both enumeration media (Figs. 5 and

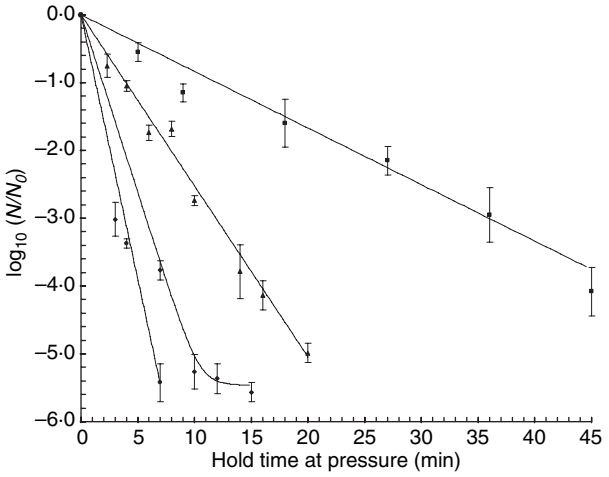

Figure 2 Survival curves of *Staphylococcus aureus* by high hydrostatic pressure in ham model system (ham/water, 4·4 : 1) enumerated in brain heart infusion (BHI) agar (■, 450 MPa; ▲, 500 MPa; ♦, 550 MPa; ●, 600 MPa). Data points are the mean values obtained from two independent experiments, each with two replicates, and error bars represent standard deviations.

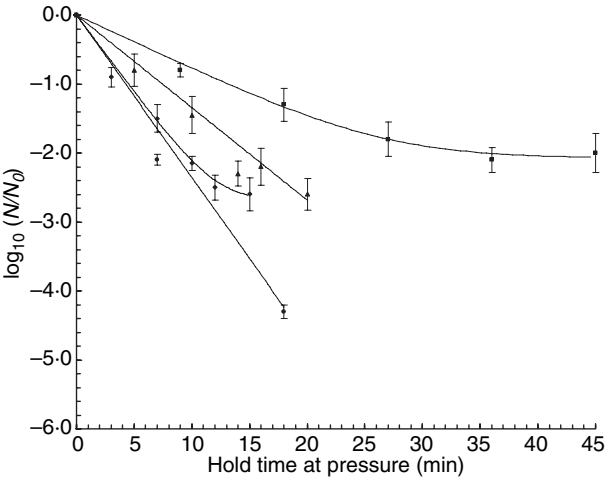

Figure 3 Survival curves of *Staphylococcus aureus* by high hydrostatic pressure in ham model system (ham/water, 4·4 : 1) enumerated in Baird Parker (BP) agar (■, 450 MPa; ▲, 500 MPa; ♦, 550 MPa; ●, 600 MPa). Data points are the mean values obtained from two independent experiments, each with two replicates, and error bars represent standard deviations.

6). For instance, a treatment at 400 MPa/55°C for 7 min reduced the population of the pathogen by 6 log CFU ml^{-1} in the nonselective medium (Fig. 5); however, extending the treatment to 10 min resulted in no further microbial reduction as tailing was evident. A similar pattern was observed for the same pressure/temperature conditions in the selective BP medium where a quick reduction of 5·2 CFU ml^{-1} was observed within the first 3 min of the processs, with no further reduction thereafter owing to prolonged tailing (Fig. 6).

Temperature played a significant role in pressure inactivation of the micro-organism; as temperature increased, the degree of inactivation also increased (Figs 5 and 6). For instance, a 10 min treatment at 400 MPa/45°C resulted in a 2·3 CFU ml^{-1} reduction in BHI agar (Fig. 5). Increasing temperature by 10°C, for the same pressure level, resulted in a 6·2 CFU ml^{-1} reduction, i.e. almost threefold inactivation of microbial cells. The positive impact of temperature is also depicted in the D_p values (Table 2). At 400 MPa/45°C, the estimated decimal reduction time was 2·15 min, whereas at 400 MPa/55°C, the relevant value was *c.* 1 min. The effect of temperature on the inactivation of *S. aureus* was even more pronounced at 450 MPa, where a direct comparison with results at ambient temperature (25°C) can be made. The estimated D_p values at ambient temperature were approximately 12 min for both enumeration media (Table 1). However, increasing temperature by 20°C resulted in a considerable reduction of the D_p values to 1·64 and 0·82 min for BHI and BP agar, respectively (Table 2).

It must be emphasized that, as the concept of decimal reduction time (D_p) presupposes log-linear inactivation kinetics, the interpretation of the values in Table 2 must be done with great caution, as there was clear evidence of deviation from linearity in the case of temperature-assisted high-pressure treatment. It is also characteristic that at 350 MPa/45°C, when the selective medium (BP) was used for the enumeration of the micro-organism, the reduction of the population was less than 1 log CFU ml^{-1} throughout the treatment (Fig. 6), and for this reason, a D_p value could not be determined (Table 2). Additionally, there was a discrepancy between the estimated D_p values at 350 MPa for the two temperatures assayed (45, 55°C). Specifically, the time needed to reduce the population of the pathogen by 1 log CFU ml^{-1} was longer at 55°C by approximately 1 min compared with 45°C (Table 2). This difference could be attributed to the curvature of the survival curve at 45°C from the onset of the treatment, thus resulting in an overestimation of the D_p value. Again, there was a difference in the recovery of the micro-organism as determined by the enumeration media (Table 2) that was in accordance with the results obtained at ambient temperature (25°C), indicating that *S. aureus* showed greater recovery in the selective BP agar than the nonselective BHI medium.

Discussion

The kinetic study of *S. aureus* at ambient temperature (25°C) in both selective (BP agar) and nonselective (BHI

Table 1 Estimated inactivation parameters of *Staphylococcus aureus* at 25°C in the pressure range 450–600 MPa suspended in a ham model system (ham/water, 4·4 : 1) and enumerated in nonselective [brain heart infusion (BHI) agar] and selective [Baird Parker (BP) agar] medium

Pressure (MPa)	Enumeration medium							
	BHI agar				BP agar			
	k_{max}* (min^{-1})	D_p† (min)	R^2	RMSE‡	k_{max} (min^{-1})	D_p (min)	R^2	RMSE
450	0·19 ± 0·01	12·1[a]	0·982	0·208	0·18 ± 0·02	12·8[b]	0·993	0·086
500	0·58 ± 0·02	3·8[a]	0·988	0·199	0·31 ± 0·03	7·4[b]	0·970	0·194
550	1·20 ± 0·29	1·9[a]	0·943	0·658	0·51 ± 0·05	4·5[b]	0·991	0·126
600	1·79 ± 0·10	1·3[a]	0·997	0·997	0·54 ± 0·06	4·3[b]	0·987	0·346
	z_p = 154 MPa				z_p = 303 MPa			

*Value ± standard error.
†D_p values with different letters within the same raw are statistically significant ($P < 0·05$).
‡Root mean square error.

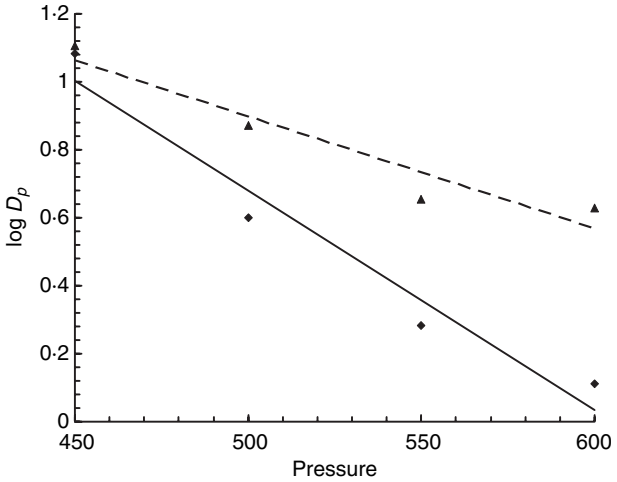

Figure 4 Pressure death time curves of *Staphylococcus aureus* by high hydrostatic pressure in ham model system (ham/water: 4·4 : 1) after enumeration in brain heart infusion agar (◆) and in Baird Parker agar (▲).

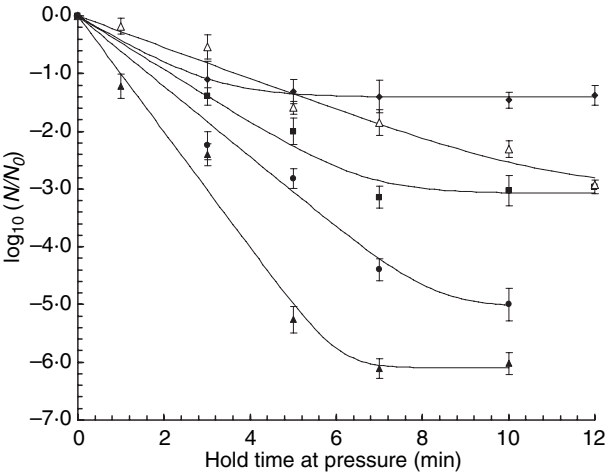

Figure 5 Survival curves of *Staphylococcus aureus* pressurized at 350, 400 and 450 MPa and different process temperatures (45, 55°C) enumerated on nonselective brain heart infusion agar (◆, 350 MPa/45°C; △, 350 MPa/55°C; ■, 400 MPa/45°C; ▲, 400 MPa/55°C; ●, 450 MPa/45°C). Data points are the mean values obtained from two independent experiments, each with two replicates, and error bars represent standard deviations.

agar) medium showed a first-order inactivation pattern with the exception of 550 MPa on BHI agar and 450/550 MPa on BP agar where a tailing effect was noticeable (Figs 2 and 3). The z_p value obtained in the selective medium was comparable with previous researchers (O'Reilly *et al.* 2000) who reported a z_p value of 359 MPa for *S. aureus* ATCC 6538 in Cheddar cheese slurry. The assumption that high-pressure inactivation of *S. aureus* follows the log-linear model is reported by other researchers (Butz and Ludwig 1986; Gervilla *et al.* 1999; O'Reilly *et al.* 2000). However, in our work, deviation from linearity (tailing) was observed, indicating that certain pressures could not considerably reduce the population of the micro-organism. This observation is in line with previous studies (Capellas *et al.* 2000) reporting that the survival curves of *Staphylococcus carnosus* treated at 500 MPa and 50°C presented a rapid drop at the beginning of the treatment followed by a long tailing thereafter. Traditionally, the inactivation of micro-organisms has been assumed to follow first-order kinetics (Raffalli *et al.* 1994; Schaffner and Labuza 1997; Ponce *et al.* 1998; Peleg 1999). However, this is hardly the case, as it was also observed in this work, and several papers have been published reporting deviation from linearity during high-pressure processing (Cole *et al.* 1993; Xiong *et al.* 1999; Chen and Hoover 2004; Buzrul *et al.* 2005; Panagou *et al.* 2007). One theory to explain the curve shape after applying moderate heat treatment suggests that the survival pattern reflects the inherent phenotypic variation in heat susceptibility of the individual vegetative cells in the population (Allwood and Russell 1970).

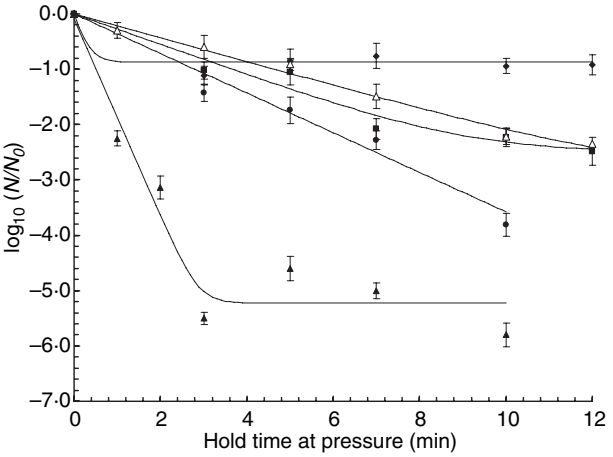

Figure 6 Survival curves of *Staphylococcus aureus* pressurized at 350, 400 and 450 MPa and different process temperatures (45, 55°C) enumerated on selective Baird Parker agar (♦, 350 MPa/45°C; △, 350 MPa/55°C; ■, 400 MPa/45°C; ▲, 400 MPa/55°C; ●, 450 MPa/45°C). Data points are the mean values obtained from two independent experiments, each with two replicates, and error bars represent standard deviations.

It is well documented that elevated temperature promotes pressure inactivation of micro-organisms (Carlez *et al.* 1993; Patterson and Kilpatrick 1998; Ponce *et al.* 1998, 1999; Stewart *et al.* 2000; Ananta *et al.* 2001). At optimal growth temperatures, inactivation is less than that at higher or lower temperatures of growth, because membrane fluidity can be more easily disrupted at temperatures beyond optimal growth (Smelt 1998). In addition, process costs of high-pressure treatment are closely related to operating pressure, hold times at pressure, maintenance and operating costs for power and labour. For this reason, it is economically beneficial to apply lower levels of pressure in combination with other processing techniques (e.g. elevated temperatures) to achieve the desired target level of microbial inactivation with a reduced processing cost (Farkas and Hoover 2000).

Comparison of our results with data from the literature is not easy as important factors of the process, such as treatment temperature, pressure come-up time, test substrate and enumeration medium are not the same. Considerable variation in pressure resistance can also be observed within strains of the same species, as food isolates are generally reported to be more pressure resistant than strains from culture collections (Cheftel 1995; Patterson *et al.* 1995; Smelt 1998; Alpas *et al.* 1999). In addition, in most published works, the survival of *S. aureus* has not been elaborated at different combinations of pressure, temperature and time, which would lead to kinetic data determination (i.e. D_p and z_p values). Usually, the pathogen is subjected to a predefined set of pressure/time treatment and the reduction in $\log_{10}$ counts is enumerated and presented. This work differs from other studies as an attempt was made to define the inactivation profile of the pathogen at different pressure/temperature regimes and estimate kinetic parameters. These data could be useful to the meat industry to design or modify existing processes without compromising on food safety. In addition, two different enumeration media were used in this study to show that the selection of the appropriate medium is crucial for the interpretation of microbial survival and should be carefully considered in similar studies.

Our results are similar to those reported by Gervilla *et al.* (1999) for *S. aureus* CECT 534 in ovine milk treated at 500 MPa for 10 and 15 min at 25°C, with reductions of approximately 2–3 log CFU ml^{-1}. For the same conditions, the reductions obtained in our work in the nonselective BHI agar were 2·5 and 3·8 log CFU ml^{-1}, respectively. In addition, our results are comparable with Guan *et al.* (2006) for *S. aureus* inactivation at 600 MPa for up to 4 min, obtaining reductions of 4·3 log CFU ml^{-1} in UHT milk. The relevant reduction obtained

Table 2 Estimated inactivation parameters of *Staphylococcus aureus* at different pressure and temperature levels suspended in a ham model system (ham/water, 4·4 : 1) and enumerated in nonselective [brain heart infusion (BHI) agar] and selective [Baird Parker (BP) agar] medium

		Enumeration medium							
		BHI agar				BP agar			
Pressure (MPa)	Temperature (°C)	k_{max}* (min^{-1})	D_p† (min)	R^2	RMSE	k_{max} (min^{-1})	D_p (min)	R^2	RMSE
350	45	0·87 ± 0·06	2·64	0·994	0·043	–‡	–	–	–
400		1·07 ± 0·15	2·15[a]	0·965	0·231	0·50 ± 0·05	4·61[b]	0·989	0·121
450		1·41 ± 0·16	1·64[a]	0·971	0·338	0·82 ± 0·16	2·81[b]	0·972	0·325
350	55	0·63 ± 0·06	3·66[a]	0·973	0·198	0·50 ± 0·03	4·6[b]	0·988	0·099
400		2·31 ± 0·25	0·99[a]	0·986	0·407	4·08 ± 0·87[b]	0·56[b]	0·947	0·593

RMSE, root mean square error.
*Value ± standard error.
†D_p values with different letters within the same raw are statistically significant ($P < 0·05$).
‡Not determined.

under the same pressure/time conditions in our experiment was 3·1 CFU ml^{-1} for the nonselective BHI medium. Patterson and Kilpatrick (1998) used high pressure against *S. aureus* NCTC 10652 in milk and poultry. Their findings showed a practical necessity for the combined use of pressure and elevated temperatures. Single-handedly, neither treatment displayed effective inactivation for the pathogen. They reported that in minced irradiation-sterilized poultry meat, *S. aureus* population exposed to 500 MPa at 50°C for 15 min was inactivated by approximately 5 log CFU ml^{-1}. However, these results are not in line with our work, as treatment at 500 MPa was carried out at ambient temperature (25°C), resulting in a reduction of *c.* 3·8 log CFU ml^{-1}. Moreover, Patterson *et al.* (1995) reported a 3 log CFU ml^{-1} reduction in the population of *S. aureus* in sterile raw poultry meat after treatment at 600 MPa/20°C for 15 min, which is again comparable with our results, as treatment of the pathogen at 600 MPa/25°C for 15 min resulted in 4·3 log CFU ml^{-1} reductions in BP agar (Fig. 3). It seems that for the same pressure level, an increase of 5°C resulted in an additional 1·3 log CFU ml^{-1} reduction in the population of the pathogen.

Using different growth media, it is possible to under/overestimate the numbers of viable bacteria, especially when cells have been damaged by physical or chemical treatments. Generally, it has been assumed that selective media do not allow for the repair of sublethally injured cells, as stressed cells are more sensitive to selective ingredients in these media (Sheridan *et al.* 1994; Taormina *et al.* 1998); it would be thus reasonable to expect higher recovery in nonselective growth media. In the present study, two different enumeration media were assayed, i.e. BP and BHI agar. The D_p values, and consequently the number of survivors, were substantially higher on the selective BP agar in the whole range of pressures applied (Tables 1 and 2). It is quite possible that the selective stimulatory action of glycine and pyruvate present in BP agar on *S. aureus* cells has overcome the destructive effect of high pressure as the cell membranes are more protected in the ham model system, hence the enhanced recovery observed in BP agar. Indeed, Park *et al.* (2001) have reported that in a food matrix, such as ham, the microbial cells are more protected against pressure effects owing to lower water activity and protein content. Moreover, fat and salt content may also have a baroprotective effect, thus preserving membrane integrity (Styles *et al.* 1991; Oxen and Knorr 1993; Palou *et al.* 1997; Garcia-Graells *et al.* 1999; Molina-Hoppner *et al.* 2004).

Overall, the results obtained in this work indicated that *S. aureus* inoculated in a ham model system was very resistant to high-pressure treatment at ambient temperature. The combined use of mild heating and lower pressure levels proved to be an effective method of reducing pathogen numbers. Moreover, as there was a difference in the enumeration of surviving cells owing to different growth media employed, i.e. selective vs. nonselective, special attention must be given to the selection of the appropriate medium to avoid underestimation of the surviving cells, as in our case, the selective medium proved to be superior in cell recovery.

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