Food Microbiology 25 (2008) 888–894

Contents lists available at ScienceDirect

Food Microbiology

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

Effect of poultry decontaminants concentration on growth kinetics for pathogenic and spoilage bacteria

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ARTICLE INFO

Article history:
Received 8 April 2008
Received in revised form
24 May 2008
Accepted 29 May 2008
Available online 14 July 2008

Keywords:
Carcass decontamination
Trisodium phosphate
Acidified sodium chlorite
Citric acid
Growth kinetic parameters
Pathogenic bacteria
Spoilage bacteria

ABSTRACT

Various chemical compounds are currently under review for final approval as poultry decontaminants in the European Union (EU). Concentration is among the factors considered by the EU authorities in the evaluation of these treatments. The aim of this research was to compare the growth parameters for pathogenic and spoilage bacteria in presence of high and low concentrations of poultry decontaminants to assess whether such treatments could involve a potential sanitary risk for consumers. Growth curves for *Salmonella enterica* serotype Enteritidis, *Listeria monocytogenes*, *Pseudomonas fluorescens* and *Brochothrix thermosphacta* were obtained at different levels of trisodium phosphate (TSP; 1.74%; 0.58%), acidified sodium chlorite (ASC; 210 ppm; 70 ppm) and citric acid (CA; 0.27%; 0.09%). The modified Gompertz equation was used as primary model to fit observed data. ASC and TSP were the most effective compounds in increasing lag phase (L) and reducing maximum growth rate (μ) in Gram-negative bacteria. Gram-positive bacteria were more influenced by CA. At high TSP levels, μ for *Salmonella* decreased. Low TSP levels increased μ for *Salmonella* and *Listeria* relative to control samples. In presence of 0.27% CA, *Brochothrix* showed the highest L and the lowest μ among strains tested. These results suggest that low TSP and high CA concentrations could favour the outgrowth of pathogenic bacteria (e.g. *Salmonella*) relative to spoilage bacteria, rending these treatments potentially dangerous for consumers. The findings of this study may be useful to the EU authorities and meat processors in their efforts to select adequate treatments for control of bacteria on poultry.

1. Introduction

At present, approximately 30% of the world's total meat consumption is poultry (FAOSTAT, 2007). This high percentage leads to great concern that a safe product of high quality should be sold. Products excessively contaminated with microorganisms are undesirable from the standpoint of public health and storage quality. At slaughtering plants in North America, it is normal practice to submit carcasses to a variety of decontaminating treatments (e.g. trisodium phosphate (TSP), acidified sodium chlorite (ASC) or organic acids) during the carcass-dressing process to reduce microbial loads. The European Union (EU) recently approved Regulation (EC) No. 853/2004 of the European Parliament and of the Council laying down specific hygienic rules on the hygiene of foodstuffs, applicable from 1 January 2006 (OJEC, 2004). This Regulation includes a procedure for the authorisation of substances other than potable or clean water to remove microbial surface contamination from foods of animal origin, hitherto prohibited for years. A number of chemical decontaminants, including TSP and ASC, are currently under review for final approval by the EU authorities. Various factors are considered in evaluating the safety and the efficacy of chemical decontamination treatments, including chemical compound concentrations (Hugas and Tsigarida, 2008).

Among the disadvantages of decontamination, it has been suggested that such procedures could render the surface of the carcass susceptible to preferential growth of dangerous bacteria because of the removal of background competitive microflora on meat (FVE, 2005; Del Río et al., 2006; Hugas and Tsigarida, 2008). Thus, the Federation of Veterinarians of Europe (FVE, 2005) recommends that the decontamination of carcasses should not be allowed unless it has been demonstrated that such techniques are safe, taking into account the potential pathogenic microflora involved.

Salmonella and *Listeria* monocytogenes are among the main pathogenic bacteria associated to poultry. On the other hand, both *Pseudomonas* and *Brochothrix* are frequently responsible for spoilage of red meat and poultry (Del Río et al., 2007a, b). It has been suggested that the capacity of *Listeria monocytogenes* to grow at low temperatures and survive in the presence of high concentrations of TSP (Gram-positive bacteria are more TSP resistant than Gram-negative bacteria) could give it a competitive

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doi:10.1016/j.fm.2008.05.011

advantage over other microorganisms and might even make TSP washes dangerous for consumers (Somers et al., 1994; Del Río et al., 2006). In previous work (Capita et al., 2001), it was observed that low TSP concentrations could favour the growth of *L. monocytogenes*. It would appear that no studies have been carried out in order to compare the behaviour of pathogenic and spoilage bacteria in the presence of different concentrations of chemical decontaminants to determine whether the outgrowth of pathogenic bacteria relative to spoilage bacteria could occur. The aim of this study is to compare the parameters of growth kinetics for pathogenic bacteria (*Salmonella enterica* serotype Enteritidis and *L. monocytogenes*) and spoilage bacteria (*Pseudomonads fluorescens* and *Brochothrix thermosphacta*) in culture broths with high and low concentrations of TSP, of ASC and of citric acid (CA), to assess whether decontaminant treatments could, in certain conditions, increase sanitary risk for consumers.

2. Methods

2.1. Strains and inocula preparation

Four strains were used in this study: *S. enterica* serotype Enteritidis CECT (Spanish Type Culture Collection) 556, *L. monocytogenes* NCTC 11994, *Ps. fluorescens* ATCC 13525 and *Br. thermosphacta* ATCC 11509. Stock cultures were kept on tryptone soy agar (TSA) slants grown for 24 h and stored at 3 ± 1 °C. To prepare the inocula, strains were subcultured twice in TSA with 0.6% yeast extract (TSA–YE) plates and incubated for 24 h at 35 ± 1 °C (*S.* Enteritidis and *L. monocytogenes*) or 25 ± 1 °C (*Ps. fluorescens* and *Br. thermosphacta*). Bacterial cells taken from single isolated colonies on TSA–YE were used to inoculate 10 ml trytone soy broth with 0.6% yeast extract (TSB–YE) and incubated, without shaking, for 18 h in order to achieve viable cell populations of 10^9 cfu/ml (Capita et al., 2001). From these cultures, decimal dilutions were prepared in peptone water for working cultures approximately 10^7 cfu/ml. All culture media and diluents were provided by Oxoid Ltd., Hampshire, UK.

2.2. Chemicals

Solutions of TSP (Merck, Darmstadt, Germany), sodium chlorite (Fluka, Madrid, Spain) acidified to pH 2.7 by adding CA (Panreac, Barcelona, Spain) (ASC), and CA were aseptically dissolved in sterile distilled water (Milli-RO, Millipore S.A., Molsheim, France) immediately before use.

2.3. Culture conditions and inoculation procedure

For assays, quantities of 135 ml of TSB–YE sterile medium ($10/9\times$) in 500 ml Erlenmeyer flasks were used. In order to simulate the pH of chicken skin, the pH was adjusted to 6.2 with 1 mol/l HCl or NaOH. Volumes of 7.5 ml of appropriate concentrations were added to flasks to give final concentrations of 1.74% and 0.58% (TSP), 210 and 70 ppm (ASC), and 0.27% and 0.09% (CA). The highest concentration tested for each compound was lower than the minimum inhibitory concentration (MIC) of the most sensitive strain, in order to allow bacterial growth. A one-third dilution was also tested for each compound to determine the effect of mild treatments on bacterial growth. The MICs were determined with the dilution broth method according to the Clinical and Laboratory Standards Institute (CLSI, 2006). The MICs observed for the strains tested are shown in Table 1. A volume of 7.5 ml of sterile distilled water was added to the untreated (control) samples. From the 10^7 cfu/ml working culture of each strain, quantities of 7.5 ml were aseptically transferred into each test flask to obtain a final concentration of approximately 5×10^5 cfu/ml. The flasks were incubated by shaking (100 strokes/min) in a Minitron orbital incubator (Biogen Científica S.L., Barcelona, Spain) at 28 °C for 48 h.

2.4. Growth and pH measurements

The concentration of bacteria and the pH were monitored before incubation (0 h) and every 2 h up to 48 h. A total of 25 analytical points were tested for each growth curve. The number of viable cells was determined by direct plating on TSA–YE using buffered peptone water for decimal dilution. The plates were incubated for 48 h at 35 ± 1 °C (*S.* Enteritidis and *L. monocytogenes*) or 25 ± 1 °C (*Ps. fluorescens* and *Br. thermosphacta*). The number of viable cells was expressed as $\log_{10}$ cfu/ml. Measurements of pH was carried out using a pH meter (Crison pHmeter Basic 20, Barcelona, Spain). Experiments were replicated six times on separate days. Duplicate measurements were taken for each replicate experiment.

2.5. Growth modelling and statistical analysis

The model used to fit growth curves to the data obtained was the modified Gompertz equation (Garthright, 1991):

$$N_t = D + A\ \exp\{-\exp[e\mu(L-t)/A+1]\}$$

where t is the time (h) that has elapsed since inoculation, $N_t = \log_{10}$ cfu/ml at time t, L the lag time (h) when the lag period ends, μ the maximum growth rate achieved ($\log_{10}$ cfu/ml/h), A the increase in bacterial concentration since inoculation to stationary phase (E) and D the upper asymptote curve (concentration of bacteria in the stationary stage, E)—A. The time to stationary phase (T) was calculated as the time elapsed to reach a concentration equal or higher than 99% of the value for E.

Values for L, μ, E and T were obtained under each set of conditions by fitting a sigmoidal curve to the data set using a Marquardt algorithm that calculates those parameter values which give the minimum residual sum of squares. The goodness of fit was evaluated using the coefficient of determination (R^2). L, μ, E and T were compared for statistical significance using analysis of variance techniques. Mean separations were obtained using Duncan's multiple range test. Significance was determined at the $P<0.05$ level. The data processing was carried out using the Statistica® 6.0 software package (Statsoft Ltd., Chicago, IL, USA).

Table 1
Minimum inhibitory concentrations (MICs) for strains tested in the presence of different chemical decontaminants

Decontaminant	*S.* Enteritidis	*L. monocytogenes*	*Ps. fluorescens*	*Br. thermosphacta*
Trisodium phosphate (%)	1.75	2.02	1.75	1.87
Acidified sodium chlorite (ppm)	250	380	220	450
Citric acid (%)	0.82	1.11	0.29	0.28

3. Results

3.1. Microbial growth curves

Including the replicates, a total of 168 growth curves representing different combinations of bacterial species, types and concentrations of chemicals were generated and fitted to the modified Gompertz equation. Twenty-five observations for bacterial densities and pH values were taken during the 48 h period for each combination. The kinetic parameters estimated from the fitted Gompertz equation are summarised in Tables 2–5. Fig. 1 shows the comparative growth curves for *S.* Enteritidis, *L. monocytogenes*, *Ps. fluorescens* and *Br. thermosphacta* in broths with different types and concentrations of chemical compounds.

The R^2 values for the Gompertz model fit were high (>0.95). Growth parameter values in control samples varied between species. The longest average L was for *Br. thermosphacta* (4.481 ± 1.054 h). This microorganism showed the lowest ($P<0.05$) densities throughout the incubation period. On the other hand, *Ps. fluorescens* had the lowest L value (1.236 ± 0.489 h). Pathogenic species had a similar lag phase. The maximum growth rate for *S.* Enteritidis (0.578 ± 0.070 h) was significantly higher than those for *L. monocytogenes* and spoilage bacteria (from 0.386 ± 0.032 to 0.443 ± 0.038 h).

ASC proved to be the most effective compound against Gram-negative bacteria, followed by TSP, these producing the highest increases in L and reductions in μ with regard to control samples. On the other hand, μ values for *L. monocytogenes* and *Br. thermosphacta* were more strongly influenced by CA. As might be expected, for most species and chemical compounds, the highest L and lowest μ values ($P<0.05$) were observed at the highest concentrations tested.

In the presence of 1.74% TSP, a similar pattern was observed for the growth of both *Salmonella* and *Listeria*. *Ps. fluorescens* appeared to be the most sensitive species, and showed the highest lag phase (5.470 ± 1.664 h) and the lowest maximum

Table 2
Lag phase (L, h) of four bacterial strains in tryptone soy broth with 0.6% yeast extract (TSB–YE) and different types and concentrations of chemical decontaminants

Decontaminant	*S.* Enteritidis	*L. monocytogenes*	*Ps. fluorescens*	*Br. thermosphacta*
Without compound	$2.599 \pm 0.362^{a}_{a}$[1]	$2.366 \pm 0.410^{a}_{a}$	$1.236 \pm 0.489^{b}_{a}$	$4.481 \pm 1.054^{c}_{a}$
TSP—1.74%	$3.643 \pm 0.464^{a}_{ab}$	$5.021 \pm 0.743^{ab}_{b}$	$5.470 \pm 1.664^{b}_{b}$	$3.971 \pm 1.117^{a}_{a}$
TSP—0.58%	$2.995 \pm 0.272^{a}_{a}$	$2.590 \pm 0.445^{a}_{a}$	$0.935 \pm 0.453^{b}_{a}$	$3.638 \pm 0.419^{c}_{a}$
ASC—210 ppm	$23.511 \pm 1.539^{a}_{c}$	$22.359 \pm 2.697^{a}_{c}$	$15.061 \pm 5.575^{b}_{c}$	$10.358 \pm 3.323^{c}_{b}$
ASC—70 ppm	$4.585 \pm 0.907^{a}_{b}$	$4.640 \pm 2.707^{a}_{b}$	$1.035 \pm 0.139^{b}_{a}$	$3.548 \pm 1.383^{a}_{a}$
CA—0.27%	$3.172 \pm 1.273^{a}_{a}$	$2.614 \pm 1.303^{a}_{a}$	$3.984 \pm 0.610^{a}_{b}$	$6.541 \pm 1.575^{b}_{c}$
CA—0.09%	$2.731 \pm 0.620^{ab}_{a}$	$2.325 \pm 0.961^{a}_{a}$	$0.990 \pm 0.511^{c}_{a}$	$3.978 \pm 1.739^{b}_{a}$

TSP, trisodium phosphate, ASC, acidified sodium chlorite, CA, citric acid.
[1] Mean ± standard deviation ($n = 6$); means in the same row with no letters in common (superscript) are significantly different ($P<0.05$); means in the same column with no letters in common (subscript) are significantly different ($P<0.05$).

Table 3
Maximum growth rate (μ; increase $\log_{10}$ cfu/ml/h) of four bacterial strains in tryptone soy broth with 0.6% yeast extract (TSB–YE) and different types and concentrations of chemical decontaminants

Decontaminant	*S.* Enteritidis	*L. monocytogenes*	*Ps. fluorescens*	*Br. thermosphacta*
Without compound	$0.578 \pm 0.070^{a}_{a}$[1]	$0.443 \pm 0.038^{b}_{a}$	$0.386 \pm 0.032^{b}_{a}$	$0.402 \pm 0.045^{b}_{ab}$
TSP—1.74%	$0.393 \pm 0.060^{a}_{b}$	$0.387 \pm 0.035^{a}_{a}$	$0.261 \pm 0.070^{b}_{b}$	$0.419 \pm 0.026^{c}_{ab}$
TSP—0.58%	$0.717 \pm 0.035^{a}_{c}$	$0.538 \pm 0.048^{b}_{b}$	$0.352 \pm 0.046^{c}_{a}$	$0.540 \pm 0.067^{b}_{a}$
ASC—210 ppm	$0.304 \pm 0.058^{a}_{d}$	$0.383 \pm 0.148^{ab}_{a}$	$0.101 \pm 0.033^{c}_{c}$	$0.529 \pm 0.197^{b}_{a}$
ASC—70 ppm	$0.220 \pm 0.013^{a}_{e}$	$0.258 \pm 0.083^{a}_{c}$	$0.212 \pm 0.019^{a}_{b}$	$0.345 \pm 0.081^{b}_{b}$
CA—0.27%	$0.491 \pm 0.040^{a}_{f}$	$0.254 \pm 0.017^{b}_{c}$	$0.463 \pm 0.060^{a}_{d}$	$0.148 \pm 0.013^{c}_{c}$
CA—0.09%	$0.567 \pm 0.129^{a}_{af}$	$0.408 \pm 0.033^{ab}_{a}$	$0.359 \pm 0.030^{b}_{a}$	$0.459 \pm 0.220^{ab}_{ab}$

TSP, trisodium phosphate, ASC, acidified sodium chlorite, CA, citric acid.
[1] Mean ± standard deviation ($n = 6$); means in the same row with no letters in common (superscript) are significantly different ($P<0.05$); means in the same column with no letters in common (subscript) are significantly different ($P<0.05$).

Table 4
Maximum population density (E; $\log_{10}$ cfu/ml) at stationary phase of four bacterial strains in tryptone soy broth with 0.6% yeast extract (TSB–YE) and different types and concentrations of chemical decontaminants

Decontaminant	*S.* Enteritidis	*L. monocytogenes*	*Ps. fluorescens*	*Br. thermosphacta*
Without compound	$9.288 \pm 0.142^{a}_{a}$[1]	$9.435 \pm 0.047^{b}_{a}$	$9.232 \pm 0.116^{a}_{a}$	$8.619 \pm 0.096^{c}_{a}$
TSP—1.74%	$9.767 \pm 0.129^{a}_{b}$	$9.264 \pm 0.180^{b}_{ab}$	$9.393 \pm 0.145^{b}_{a}$	$8.998 \pm 0.274^{c}_{b}$
TSP—0.58%	$9.639 \pm 0.073^{a}_{bc}$	$9.481 \pm 0.067^{b}_{a}$	$9.330 \pm 0.223^{b}_{a}$	$8.923 \pm 0.074^{c}_{b}$
ASC—210 ppm	$9.603 \pm 0.322^{a}_{bc}$	$9.457 \pm 0.379^{a}_{a}$	$8.285 \pm 0.773^{b}_{b}$	$8.473 \pm 0.052^{b}_{ac}$
ASC—70 ppm	$9.371 \pm 0.190^{a}_{ac}$	$9.454 \pm 0.096^{a}_{a}$	$9.108 \pm 0.112^{b}_{a}$	$8.547 \pm 0.107^{c}_{ac}$
CA—0.27%	$8.821 \pm 0.399^{a}_{d}$	$9.196 \pm 0.049^{b}_{b}$	$8.996 \pm 0.075^{ab}_{a}$	$8.166 \pm 0.041^{c}_{d}$
CA—0.09%	$9.136 \pm 0.124^{a}_{a}$	$9.321 \pm 0.027^{b}_{ab}$	$9.264 \pm 0.206^{ab}_{a}$	$8.405 \pm 0.070^{c}_{c}$

TSP, trisodium phosphate, ASC, acidified sodium chlorite, CA, citric acid.
[1] Mean ± standard deviation ($n = 6$); means in the same row with no letters in common (superscript) are significantly different ($P<0.05$); means in the same column with no letters in common (subscript) are significantly different ($P<0.05$).

Table 5
Time to stationary phase (T, h) of four bacterial strains in tryptone soy broth with 0.6% yeast extract (TSB–YE) and different types and concentrations of chemical decontaminants

Decontaminant	*S.* Enteritidis	*L. monocytogenes*	*Ps. fluorescens*	*Br. thermosphacta*
Without compound	$14.667 \pm 1.033^{a1}_{a}$	$18.333 \pm 0.816^{b}_{a}$	$20.667 \pm 2.066^{c}_{ab}$	$20.333 \pm 0.816^{c}_{ab}$
TSP—1.74%	$24.000 \pm 2.828^{a}_{b}$	$20.667 \pm 2.422^{a}_{a}$	$34.000 \pm 5.657^{b}_{c}$	$19.333 \pm 3.266^{a}_{a}$
TSP—0.58%	$13.667 \pm 0.816^{a}_{a}$	$16.000 \pm 1.265^{b}_{a}$	$23.667 \pm 2.944^{c}_{a}$	$16.000 \pm 1.265^{b}_{c}$
ASC—210 ppm	$45.333 \pm 2.733^{a}_{c}$	$41.000 \pm 6.782^{a}_{b}$	$44.333 \pm 5.125^{a}_{d}$	$22.667 \pm 3.011^{b}_{b}$
ASC—70 ppm	$35.330 \pm 1.633^{a}_{d}$	$31.667 \pm 6.377^{a}_{c}$	$35.000 \pm 2.757^{a}_{c}$	$22.667 \pm 3.266^{b}_{b}$
CA—0.27%	$15.667 \pm 1.966^{a}_{a}$	$27.667 \pm 1.506^{b}_{c}$	$19.000 \pm 2.098^{c}_{b}$	$34.333 \pm 0.816^{d}_{d}$
CA—0.09%	$15.333 \pm 3.011^{a}_{a}$	$19.000 \pm 2.449^{bc}_{a}$	$21.667 \pm 2.338^{b}_{ab}$	$17.333 \pm 2.266^{ac}_{ac}$

TSP, trisodium phosphate, ASC, acidified sodium chlorite, CA, citric acid.
[1] Mean ± standard deviation ($n = 6$); means in the same row with no letters in common (superscript) are significantly different ($P < 0.05$); means in the same column with no letters in common (subscript) are significantly different ($P < 0.05$).

growth rate (0.261 ± 0.070 $\log_{10}$ cfu/ml/h) among the four species. *Br. thermospacta* showed the highest growth rate (0.419 ± 0.026 $\log_{10}$ cfu/ml/h), as well as the highest population densities from 6 to 14 h after incubation, of all the species of bacteria under test. Low TSP concentrations (0.58%) increased μ relative to control samples of both the pathogenic bacteria (Table 3), this leading the concentration of such bacteria to be higher than that of spoilage bacteria throughout the incubation period. Low TSP concentrations did not affect significantly the growth of spoilage bacteria, whose rates were comparable to those of the control samples.

For all four strains, the presence of high ASC concentrations in the growth medium significantly increased the lag phase with respect to control samples. This compound decreased the maximum growth rate only for Gram-negative bacteria. *Pseudomonas* exhibited the lowest (0.101 ± 0.033 $\log_{10}$ cfu/ml/h), and *Brochothrix* the highest (0.525 ± 0.197 $\log_{10}$ cfu/ml/h) maximum growth rate among the strains tested. The time to stationary phase of this bacterium was half that of the other three species. Low ASC concentrations were effective in reducing the maximum growth rate of all four bacteria relative to controls (Table 3).

In the presence of high CA concentrations *Br. thermospacta* showed the highest L (6.541 ± 1.575 h) and T (34.333 ± 0.816 h), as well as the lowest μ (0.148 ± 0.013 $\log_{10}$ cfu/ml/h), of all the four bacteria tested. The presence of low CA concentrations do not modified the growth parameters (L and μ), with regard to control samples, of strains tested.

3.2. pH evolution

As was to be expected, broths with TSP had the highest, and those with CA the lowest, initial pH values (Fig. 2). The pH of samples inoculated with *S.* Enteritidis, *L. monocytogenes* or *Br. thermosphacta* decreased during the first few hours of incubation and then remained relatively constant throughout the period of study. Samples inoculated with *Ps. fluorescens* remained almost unchanged throughout incubation. At the end of the incubation period the pH of broths with TSP remained higher than those of broths with ASC or CA and those without decontaminants.

4. Discussion

4.1. Microbial growth curves

The modified Gompertz equation was regarded as adequate for describing *in vitro* bacterial growth in the presence of chemical decontaminants because of the good agreement observed between experimental and predicted values.

The higher growth rate observed in control samples for pathogenic bacteria (especially *Salmonella*), as against spoilage bacteria, coincides with previous observations in poultry artificially co-inoculated with *L. monocytogenes* and *Br. Thermosphacta* (Del Río et al., 2006). This suggests that from the standpoint of public health it would be advisable to use antimicrobials in order to modify this pattern and prevent outgrowth of pathogenic bacteria.

The antimicrobial activity of the decontaminants tested has been previously demonstrated both *in vitro* and on red meat and poultry carcasses (Del Río et al., 2007a). In a previous investigation carried out on chicken legs (Del Río et al., 2007a), it was observed, as in the research being reported here, that ASC and TSP were the most effective antimicrobial agents for Gram-negative pathogenic bacteria. On the other hand, in the above mentioned research, CA and TSP showed the highest effectiveness against Gram-positive species.

The antimicrobial action of ASC is attributed to chlorous acid, which derives from the conversion of chlorite ions into an acid form under acidic conditions. Chlorous acid kills microorganisms by direct action on the cell membrane and by the oxidation of cell constituents (Castillo et al., 1999; SCVPH, 2003). The low pH of ASC solutions is also partially responsible for the antimicrobial action of ASC.

The antimicrobial effect of TSP *in vitro* results from a combination of several factors. First, there is the high pH (12–13) of TSP solutions, which appears to disrupt fatty molecules in the cell membrane, causing the bacterial cells to leak intracellular fluid. Second, there is its ionic strength, which can cause bacterial cell autolysis. The ability to remove fat films and to have a surfactant or detergent effect also contributes to the decontaminant effect of TSP on red meat and poultry carcasses (Capita et al., 2002).

The bactericidal effect of organic acids, such as CA, is due to the reduction in pH to below the growth range for bacteria, and metabolic inhibition by undissociated molecules which penetrate their cell membranes. The accumulation of undissociated weak acid in the cytoplasm of the cell eventually leads to the acidification of the microorganism's cytoplasm (Booth, 1985).

The striking increases in lag time and decreases in the maximum growth rate observed in the presence of strong concentrations of the chemicals used in the present study would appear to be related to the extreme pH which these concentrations cause in the culture medium, as previously reported (Del Río et al., 2007b). The great susceptibility of *Pseudomonas* to 1.74% TSP observed in research being presented here is in agreement with observations of other authors (Colin and Salvat, 1996; Ellerbroek

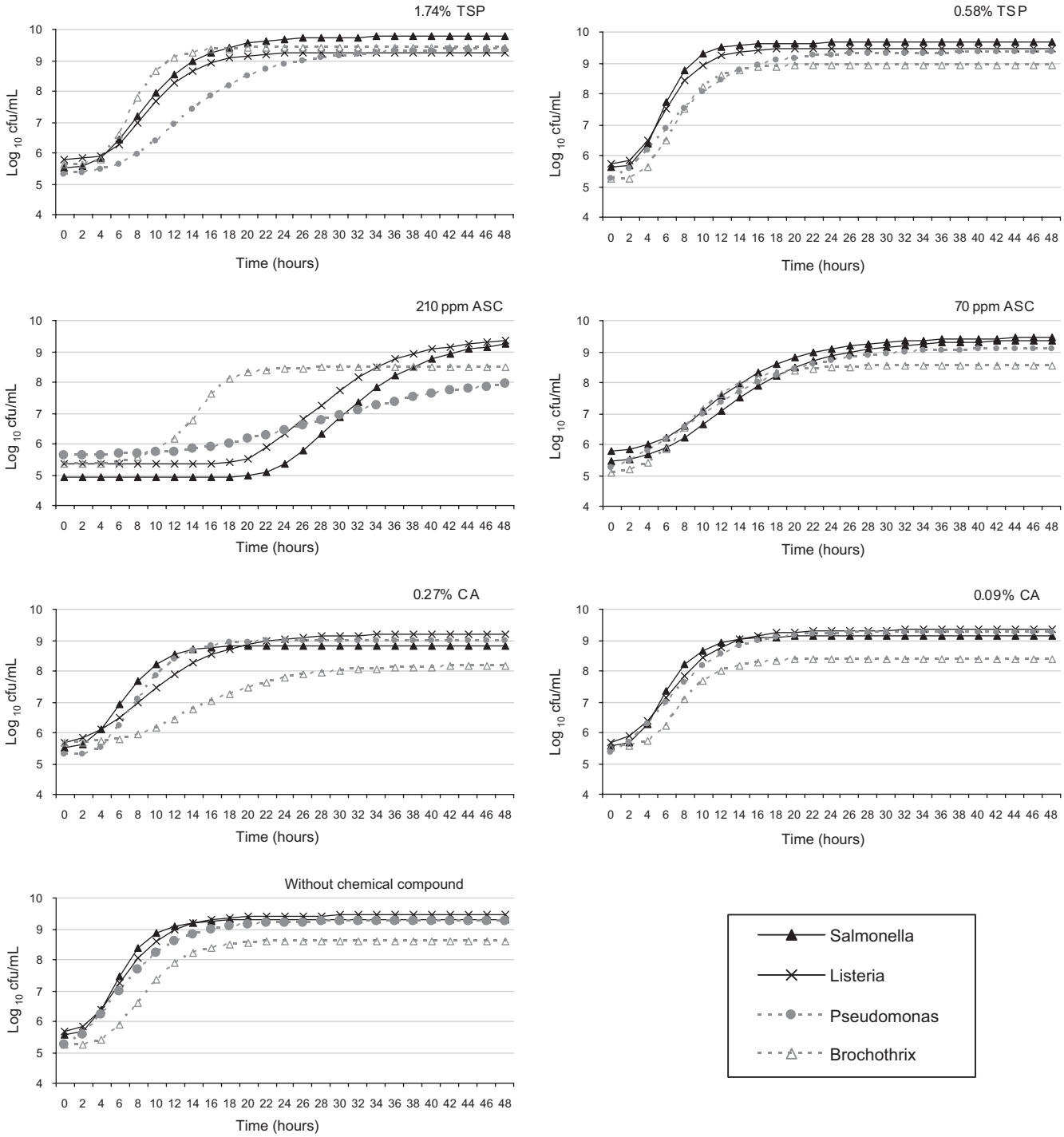

Fig. 1. Growth curves for pathogenic and spoilage bacteria in the presence of different types and concentrations of decontaminants (modified Gompertz fits). TSP, trisodium phosphate; ASC, acidified sodium chlorite; CA, citric acid.

et al., 1997; Dorsa et al., 1998). Somers et al. (1994) suggest that the very limited resistance of *Pseudomonas* to TSP, combined with the capacity of some Gram-positive pathogenic bacteria (like *L. monocytogenes*) to grow at low temperatures and survive in the presence of strong concentrations of TSP (Gram-positive bacteria are more TSP resistant than Gram-negative bacteria; Capita et al., 2002; Sampathkumar et al., 2003) could give it a competitive advantage over other microorganisms, and might even render TSP washes dangerous if raw material is contaminated. However, results in the present study show that growth rate of *Br. thermosphacta* was higher than those of pathogenic bacteria, as reported by other authors (Colin and Salvat, 1996; Salvat et al., 1997). These findings suggest that there is no risk of the emergence of pathogenic over spoilage bacteria as the result of treatments with high concentrations of TSP.

The higher maximum growth rate of *Salmonella* and *Listeria* achieved in the presence of 0.58% TSP might be explained by taking into account the initial pH of the broth. Concentrations of 0.58% TSP caused a pH of approximately 7.5 in TSB–YE with an initial pH of 6.2, this change being favourable for the growth of such bacteria. In the absence of TSP, the pH of the broth (6.2) is also suitable, though not optimal, for their growth. These results confirm previous

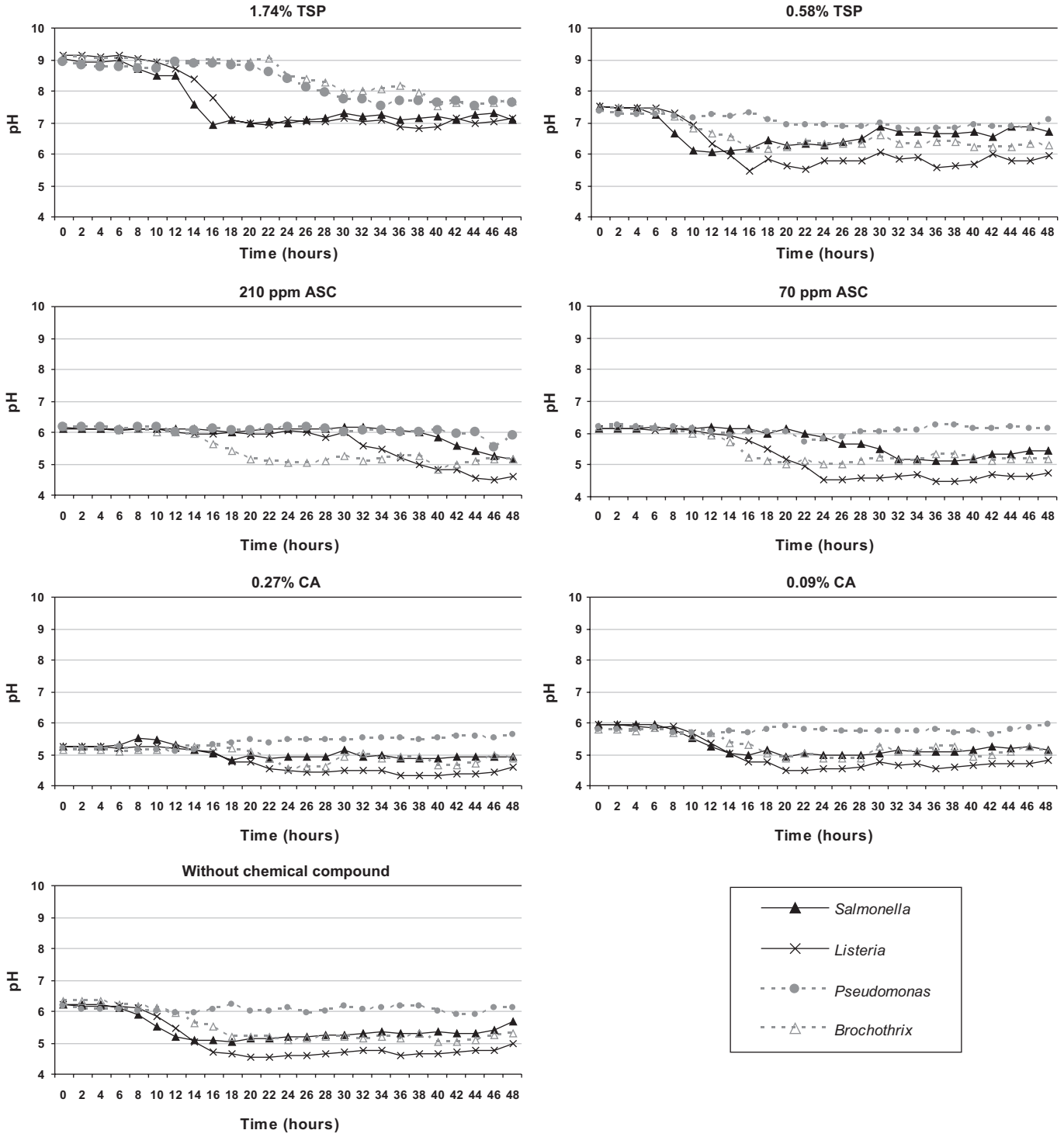

Fig. 2. Changes in pH in broths with different bacterial species, types and concentrations of chemical compounds. TSP, trisodium phosphate; ASC, acidified sodium chlorite; CA, citric acid.

findings with *L. monocytogenes* and 0.5% TSP (Capita et al., 2001) and suggest that low TSP concentrations could favour the growth of pathogenic bacteria, which makes its use unadvisable.

As in the case of treatment with a strong concentration of TSP, ASC at 210 ppm favoured the overgrowth of *Brochothrix* among the species tested, suggesting that in this case spoilage by this bacterium would occur prior to any noteworthy increase in the levels of the pathogenic microorganisms. The increased lag phase and decreased growth rate of *Brochothrix* in the presence of CA might potentially pose a risk for consumers of treated products contaminated with *Salmonella* and packed in absence of oxygen, since this do not allow the growth of aerobic spoilage bacteria like *Pseudomonas*. Hence, some pathogenic bacteria might multiply to appreciable levels before spoilage by *Brochothrix* occurred.

4.2. Changes in pH

With respect to pH values, the results being reported here coincide with those of other authors (Kanellos and Burriel, 2005;

Mehyar et al., 2005), who found that of all the chemicals tested TSP caused the largest initial increase in pH, while organic acids had the opposite effect. The low pH observed in ASC-treated samples may be due to the presence of CA, used to acidify the sodium chlorite (Lim and Mustapha, 2004).

The coincidence between the start of the exponential growth phase and the beginning of a drop in pH for the majority of combinations of chemicals and bacteria investigated could be due to the acids formed as a consequence of the use of glucose during microbial growth (Mu et al., 1997; Capita et al., 2001). In fact, during the lag phase and the stationary phase pH remained virtually constant. Chemical compounds formed from the decontaminants could be also responsible for this fall in pH values (Su and Morrisey, 2003).

The fact that on day 5 of storage, the highest pH was observed in samples treated with TSP and the lowest in those treated with CA is a finding that coincides with the observations of other authors investigating poultry (Gill and Badoni, 2004).

5. Conclusions

To sum up, ASC showed the greatest ability to increase the lag phase and reduce the growth rate of the bacteria tested. TSP showed the second best capacity against Gram-negative bacteria, and CA against Gram-positive species. It was observed that its concentration is a fundamental aspect to be kept in mind when using a decontaminant. In the presence of strong concentrations of TSP or ASC, *Br. thermosphacta* showed the highest growth rate among bacteria tested, suggesting that spoilage could occur prior to any marked increase in the levels of the pathogenic microorganisms. On the other hand, low levels of TSP actually increased the growth rate of *S.* Enteritidis and *L. monocytogenes*, rendering this treatment potentially dangerous for consumers because a marked increase in the level of pathogenic bacteria might take place before spoilage. The strong antimicrobial effects of CA against *Br. thermosphacta* suggest a potential for the overgrowing of pathogenic, relative to spoilage, bacteria in anaerobically packed products. It must be pointed out that results in the present paper should be considered with caution because of the *in vitro* experimental conditions for growth comparison. Thus, further studies are necessary in order to confirm these findings in detail.

Acknowledgements

This study was given financial support by the Spanish *Ministerio de Sanidad y Consumo* (*Instituto de Salud Carlos III*, FIS PI 040722).

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