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Effect of superheated steam on the inactivation of *Listeria innocua* surface-inoculated onto chicken skin

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Abstract

This study investigated the effectiveness of superheated steam in the decontamination of poultry skin. The steam jet was at a temperature of 160 °C and can be superheated up to 500 °C, leading to an impinging jet temperature of 400–450 °C. Disks of poultry skin were surface-inoculated with about 10^7 CFU/cm^2 *Listeria innocua* (CLIP 20595) and treated with either superheated or non-superheated steam for up to 60 s. Surface temperature was carefully measured throughout the treatment, and results were analyzed using a previously developed transfer model. The overall pattern of *L. innocua* inactivation was displaying an initial pattern of rapid decline followed by much slower decline thereafter. Superheated steam was clearly more bacterial inactivation-efficient than non-superheated steam, leading to an average reduction of more than 5log$_{10}$ CFU/cm^2 after 30 s of treatment. Large variations in surviving cell numbers were observed between replicates which cannot be explained by variations in the heat treatment.

Keywords: Thermal decontamination; Superheated steam; Poultry skin; *Listeria*

1. Introduction

There has been renewed interest in thermal decontamination of the surface of food products due to both consumer demand for safer products and consumer dislike of the use of chemicals and irradiation. In the poultry industry, bacteria adhering to the skin and carcass cavity are the main sources of contamination of the underlying tissues during portioning, skinning and boning. There is a trend towards increased contamination problems since carcasses are increasingly being processed into value-added convenience pieces and the processing rates are higher. Moreover, European legislation on pathogens contamination is becoming increasingly restrictive. A decontamination intervention which would eliminate pathogenic bacteria from the skin of chicken and turkey carcasses would be an efficient way to break the chain of cross-contamination from live animal to processed meat products (Avens et al., 2002).

Thermal decontamination of food surfaces can be commonly obtained using hot air, hot water immersion, or flowing steam. Water immersion and steam successfully decontaminate the food surface (Avens et al., 2002; James and James, 1997; James et al., 2000) while hot air has proved to be less efficient (James and Evans, 2006). For any given period, the efficiency of a heat treatment tends to increase with temperature. Decontamination treatments have been developed using pressurized steam, but these systems have very short treatment times that appear to achieve only limited microbial inactivation (Kozempel et al., 2001; Morgan et al., 1996).

The inactivation of bacteria or spores by superheated steam has seldom been studied, and only under temperature conditions below or approaching 100 °C (Spicher et al., 2002). However, superheated steam has been widely used for the drying of biological or non-biological products, including foods (Braud et al., 2001; Moreira, 2001;

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Taechapairoj et al., 2006), wood chips (Fyhr and Rasmuson, 1997) or even coal (Chen et al., 2000). The present paper compares *Listeria innocua* inactivation efficiency between a high temperature superheated steam treatment and a non-superheated steam treatment.

The strain *L. innocua* CLIP 20595 isolated from meat was chosen for its non-pathogenicity and because its growth properties approach the same rates as an *L. monocytogenes* strain in a broad field of a_w, pH levels and temperatures (Begot et al., 1997). This behavioral similarity has been confirmed in other meat tryptone broth (Lebert et al., 1998) and beef meat surface studies (Robles Olvera et al., 1999). The strain was isolated from a meat processing workshop and therefore theoretically presents a stronger decontamination resistance than clinical strains or laboratory strains that date back a while.

Initial rapid linear decrease in pathogen numbers followed by a 'tailing' period, is the pattern generally observed during inactivation of food and abiotic surfaces by air or steam-heating systems (Gaze et al., 2006; McCann and McGovern et al., 2006; McCann and Sheridan et al., 2006; Valdramidis et al., in press). These systems also display very large variations among replicates. This variation is generally interpreted as a result of changes in the test apparatus conditions, but these changing conditions remain largely unknown since product surface temperature is seldom recorded and analyzed. This situation makes it difficult to compare and interpret results and leads to major uncertainty in model predictions (Valdramidis et al., 2006, in press). In this study, variations in product surface temperature were carefully measured and interpreted based on previous analyses of the heat exchanges between product and superheated steam jet (Kondjoyan and Portanguen, in press). Experimental points were abundantly repeated and analyzed to yield reliable results.

2. Materials and methods

2.1. Inocula – sample preparation and inoculation

Isolates of *L. innocua* CLIP 20595 were stored on cryoprotective beads (Mast Diagnostic, France) in a freezer at −18 °C. Isolates were transferred onto a TSA gel slide and left for 8 h at 37 °C for recovery adaptation. Then two "loops of platinum wire" of the culture were transferred to 100 mL of meat broth (10 g/L meat peptones from Merck, Germany; 5 g/L yeast extracts from Difco, USA; 5 g/L glucose from Prolabo, France; 40 g/L tryptic soy agar, TSA, from Difco, USA), and the bacteria were incubated at 20 °C until they entered the late stationary phase of growth, i.e., for 20 h. This suspension was used to directly inoculate chicken skin samples.

The chicken skins used were taken from the upper legs of supermarket chickens from the same slaughterhouse and marketed under the same name. All meat samples were stored at 4 °C until use. Eleven cm^2 of skin were excised under sterile conditions before each experiment. Thickness of the poultry skin was measured before each experiment. The skin was placed between two flat plastic plates of a precisely determined thickness. Skin thickness was measured to an accuracy of ±0.1 mm, but as the strip of skin was not uniform the measurement only gave an average value of its thickness. The skin was then set on a 20 mm thick, 50 mm diameter Teflon® brace. The skin disk was held in place using a sterile rubber fitted to a groove so that the whole assembly can be set into a sample-holder. The outer skin surface was then placed on a plastic grid and immersed (outer face down) in the bacterial suspension for 60 seconds. The skin was then left to drain for 10 min at room temperature on an identical grid.

2.2. Heat treatment apparatus and temperature measurement

A detailed description of the experimental apparatus and analysis of its functioning have been given by Kondjoyan and Portanguen (in press). Steam was produced by a generator operating under 5–7 bars of pressure (steam temperature, 150–165 °C) and was released into a stainless steel pipe channeling the steam to the sample (Fig. 1). Superheated steam was obtained via electrical resistances heating the pipe. A straight pipe section was then bent to position the outlet just above the sample surface. During these experiments, the distance d between the sample surface and the pipe outlet was set at 36 mm. Sample and support were positioned on a sliding device mounted on a ball bearing system. The temperature at the sample surface was measured using a digital Infra-Red pyrometer (IN 500, Impact system) over a spot of 20 mm in diameter located at the center of sample. The measuring part of the Infra-Red system was attached to the sliding device to be able to measure the sample surface temperature throughout the decontamination treatment. Temperature of the impinging jet was measured every second using a 0.5 mm thick calibrated type K thermocouple positioned 3.0 mm above the middle of the sample surface. Before each experiment, the axis of the pipe was centered at the middle of the sample surface. The measurement spot of the IR thermometer was centered at the same point using a laser beam. The generator was switched on, and the temperature of the heating pipe was set to obtain the desired jet temperature at the sample surface. The apparatus was kept under the same heating conditions for at least four hours so as to reach steady-state conditions before the experiments began. The sample was moved 300 mm away from the superheated steam jet, after which steam was released for a few minutes to purge the system and to limit droplet formation in the jet. The sample was then slid beneath the jet. At the end of the heat treatment, the sample surface was rapidly cooled by sliding the sample beneath a 45–55 m s^{-1} jet flow of cold air (temperature 3–5 °C) produced by a Ranque–Hilsch tube ("vortex tube"). The three positions of the sliding system, i.e., sample away from the superheated steam jet, sample subjected to jet, and sample subjected to cold air, were set perfectly using a blocking ball bearing system.

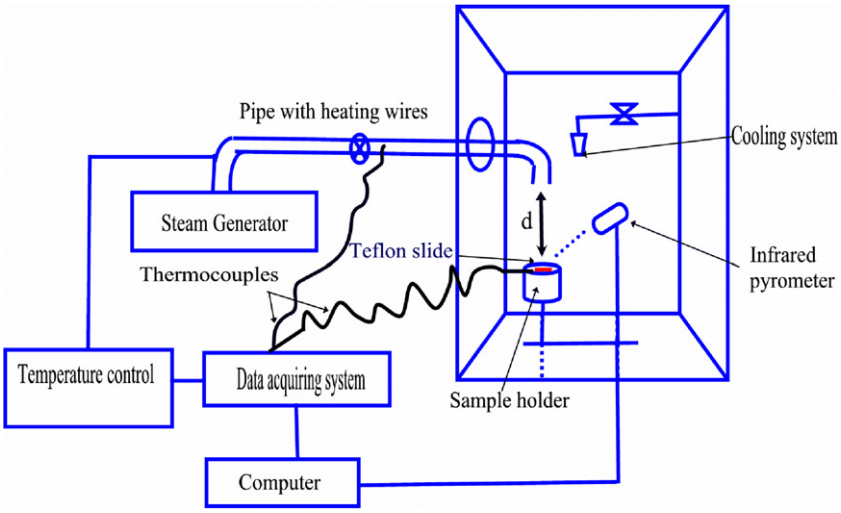

Fig. 1. Schematic representation of the superheated steam decontamination rig and its main components designed by QuaPA-C2T.

The operation of the device, the characteristics of the superheated steam jet and the surface temperature of the sample have been analyzed in depth, as reported in a previous article (Kondjoyan and Portanguen, in press). This work made it possible to correct measurement bias related to the fact that the superheated steam jet was non-transparent to the infrared radiations. It also yielded an in-depth analysis of the repeatability and reproducibility of the heat treatment at the sample surface. It was demonstrated that the temperature of the superheated steam jet could vary in relation to larger or smaller quantities of condensates on the internal walls of the tube, but that this variation was limited to just a few dozen degrees at a tube superheat temperature of 500 °C. The difference in sample surface temperatures was determined in the central 20 mm diameter area where the microbiological samples had been taken. The standard deviation in surface temperature in this area never went above 10 °C at a jet temperature of 200 °C, despite the fact that greater temperature differences could be observed in the jet itself.

2.3. Enumeration of surviving bacteria

After the heat treatment, a 20 mm diameter skin disk (3.5 cm^2) was cut away from the center of the sample-holder (the IR pyrometer spot area). The skin disk was then placed in a stomacher bag containing 35 mL of physiological saline solution and ground for 60 s. One milliliter of this suspension was successively diluted 1:10 in physiological saline solution until the required estimated bacterial concentration was reached. Enumeration was carried out either using a spiral plater (Interscience, France) and a small volume of inocula or by counting the colonies which came from the insertion of the inocula in the bulk of the culture medium during jellification. This latter technique enabled the introduction of a bigger volume of inocula in the culture medium and was used when the bacterial concentration of the inocula was very low (very efficient decontamination treatments). In both cases, the culture medium used was a *Listeria*-selective Palcam medium (Palcam, Merck, Germany). For spiral plater bacterial enumeration, 49.2 μL of suspension was spread over the agar gel, and the detection threshold was 5×10^2 CFU/cm^2. When the heat treatments caused greater bacterial destruction, this detection threshold was lowered by increasing the volume of inoculate. Then, either 1 or 2 mL volumes of suspension were directly inoculated in the bulk of the gel, which led to detection thresholds of 8.5×10^1 and 4.3×10^1 CFU/cm^2, respectively.

2.4. Experimental design

Inactivation of bacteria was observed by McCann and Sheridan et al. (2006) during the steam pasteurization of poultry and other meat samples. In all cases, pathogen numbers declined rapidly during the first 10–30 s of steam treatment, followed by a 'tailing' period. Thus, our measures of bacterial inactivation were concentrated on the first declining period which included three treatment times: 10, 20, 30 s while only one treatment time of 45 or 60 s was used to characterize the tailing period. The 60 s duration was only used under the non-superheated steam conditions. When superheated steam was used, the 60 s treatment led to significant deterioration of the skin. Thus, in this case the longest treatment duration was capped at 45 s.

Inactivation results obtained on surface decontamination of foods are highly scattered (Gaze et al., 2006; McCann and McGovern et al., 2006; McCann and Sheridan et al., 2006; Valdramidis et al., 2006; Valdramidis et al., in press). More often than not, the experiments are only repeated on three separate occasions. In this study, we considered that this was not sufficient to accurately compare bacterial inactivation effects between superheated steam and non-superheated steam. Thus, experiments were

repeated on ten separate occasions for the following treatment times: 10, 20, and 45 or 60 s, and on 20 separates occasions for the 30 s treatment, which marks midway through the longest treatment duration.

Following McCann and Sheridan et al. (2006), the relationships between pathogen numbers and treatment times were fitted using the following non-linear regression:

$$Y = A + BR^t \quad (1)$$

where Y pathogen is counted in $\log_{10}$ units, t is the time in seconds, and A, B and R are parameters that must be estimated. The asymptote of the model is A, the count at time zero is $A + B$, and the parameter R influences the rate of decay. In this model R shall be constrained between -1.0 and -1.0 to avoid Y to go to infinity when time increases. Values of A, B, and R were determined by minimizing the sum of the squares of deviation between the experimental values and those calculated by (1). The "lsqCurvefit function" of Matlab7.0 was used and $A + B$ was constrained to be equal to the $\log_{10}$ of the initial level of the microbial population.

Relation (1) can be used to interpolate values between experimental measurement times but it does not rely on any backstage microbiological consideration. Thus other inactivation models were tested using the GInaFIT (Geeraerd et al., 2005).

3. Results

3.1. Temperature measurements

During the decontamination experiments, the temperature of the steam delivery tube was set at either 200 or 500 °C, giving jet temperatures of 160–180 °C and 400–450 °C, respectively. The typical calibrated temperatures measured at the surface of the chicken skin samples under both types treatment are given in Fig. 2 for illustrative purposes. The sample surface heat-up progressed over three periods, i.e., (1) initial steam condensation, (2) evaporation of condensates, and (3) heating of sample surface by convection. The last treatment period was the rapid cooling of sample surface by the cold air jet. Under the 200 °C steam jet treatment, there was no third convection heat exchange period, and decontamination was carried out at a surface temperature which was equal to the temperature of the boiling water at 850 m altitude. This value was measured to be 96 ± 2 °C for all the non superheated steam experiments. In contrast, under the 500 °C superheated steam jet treatment there was no visible period of condensate evaporation. The rapid rise in temperature due to steam condensation from the initial product temperature to 96 °C was followed by a period where the surface was convection-heated. During this period the surface temperature rises up to a maximum value. This maximum value was higher for longer thermal treatment. Surface temperatures were not perfectly repeatable from one experiment to another and complex temperature variation patterns were observed due to the skin cooking and denaturing processes. Average and standard deviation of the mean and maximum temperature during the convection-heating period calculated on all superheated steam experiments are given in Table 1. Standard deviation is always less than 10% of the measured value except for the 10 s treatment where more variations is observed. Simulations have shown that in this case, the surface temperature increase over the first 5–10 s of convection phase is extremely sensitive to the thickness of the chicken skin (Kondjoyan and Portanguen, in press). The superheated steam heating (a 500 °C tube temperature) resulted in end-of-treatment chicken skin surface temperatures which can be very important for the longest treatments. The maximum surface temperature during the 45 s experiments was on the average 218 °C and could reach 240 °C in some cases.

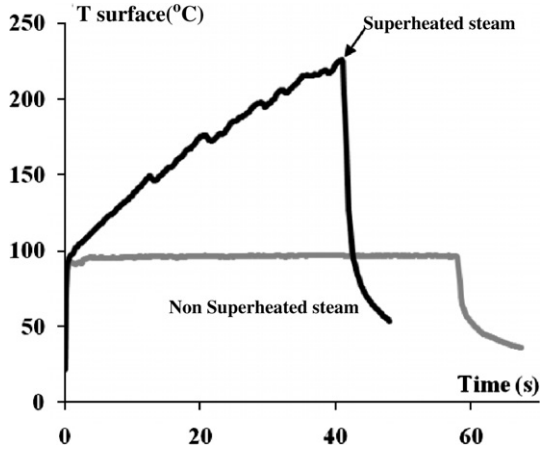

Fig. 2. Typical changes in surface temperature measured by the calibrated IR pyrometer at the surface of the poultry skin for the superheated steam and the non-superheated steam treatments, respectively. Jet temperatures measured at 3 mm above the sample were 165 and 434 °C for the non-superheated and the superheated treatments, respectively.

3.2. Microbial survival after the steam treatments

3.2.1. Inactivation for a surface temperature of 96 °C

Fig. 3 reports the average *L. innocua* population change patterns for a surface temperature of 96 °C with a standard deviation ranging between 1.1 and 1.5 $\log_{10}$, regardless of treatment time (Table 2). The standard deviation figures did not change significantly when the experiment was

Table 1
Average and maximum temperatures reached at different times of the superheated steam treatment; standard deviations (in °C and in %) on these values

Time (s)	$T_{s\ mean}$	SD	SD	$T_{s\ max}$	SD	SD
	Average (°C)	(°C)	(%)	Average (°C)	(°C)	(%)
10	121	11	9	140	20	14
20	140	8	6	171	11	6
30	149	13	9	183	18	10
45	175	10	6	218	19	9

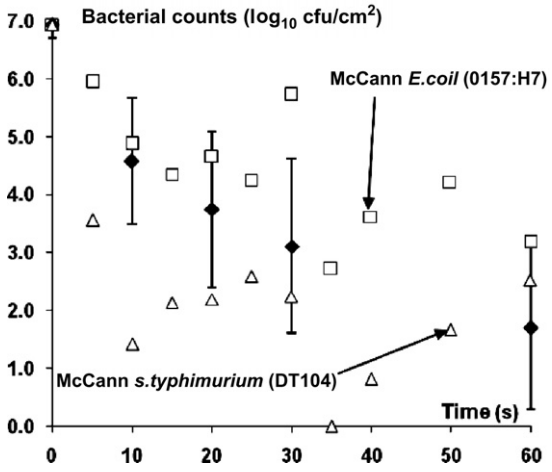

Fig. 3. Relationship between the survival of *Listeria innocua* (CLIP 20595) and time on the poultry skin following the non-superheated steam treatment (black diamonds). Comparison with the results on the inactivation of *E. coli* O157:H7 (white squares) and *S. typhimurium* DT104 (white triangles) obtained on poultry skins by McCann and Sheridan et al. (2006).

Table 2
Average, maximum and minimum *Listeria innocua* (CLIP 20595) populations measured at different treatment times for the 96 °C treatment; standard deviations on the experimental values

Time (s)	Mean pop.	Max pop.	Min pop.	SD
0	6.9	7.4	6.7	0.2
10	4.6	6.1	2.3	1.1
20	3.8	5.3	1.7	1.3
30	3.1	5.7	0.0	1.5
60	1.7	3.6	0.0	1.4

repeated 20 times at a 30-s heat period instead of 10 times for the other treatment periods. This tended to show that in principle, 10 repetitions are enough to give a good idea of averages and scatter. Furthermore, 90% of the measurements fell within an interval that was twofold higher than the standard deviation, and the median value of the reduction in microbial population was very similar to the average, which tends to highlight a random results distribution. During McCann's experiments (McCann and Sheridan et al., 2006) results were very scattered especially on chicken skin where the standard deviation between replicates was: $\pm 1.98\ \log_{10}$ (CFU/cm^2) and $2.37\ \log_{10}$ (CFU/cm^2) for *Escherichia coli* and *Salmonella typhimurium* respectively. Systematically repeating the experiments confirmed extremely scattered results for microbial inactivation at the surface of poultry skin, even under the extremely well-controlled heat treatment conditions obtained in this work during the non-superheated steam treatment (constant temperature of 96 ± 2 °C). Extreme microbial population's values given in Table 2 are systematically found whatever the treatment durations and cannot be explained by biases in the microbial procedure neither by difference in the thermal treatment. MacCann's experimental points have been reported in Fig. 3 to be compared to the present results obtained on *L. innocua* CLIP 20595. The reduction in *L. innocua* populations are on a similar scale to the reduction of *E. coli* O157:H7 and *S. typhimurium* DT104 populations observed by McCann and Sheridan et al. (2006) on chicken skin surfaces subjected to steam/air mixtures of around 87 °C (for an estimated chicken skin surface temperature of 83–84 °C). These surface temperature differences and the variability in experiments preclude any further discussion of the respective heat-resistant properties of *L. innocua* CLIP 20595, *E. coli* O157:H7 and *S. typhimurium* DT104.

3.2.2. Inactivation under the superheated steam treatment

Fig. 4 reports the average *L. innocua* population change patterns for a superheated steam treatment. The standard deviation of the measurements ranged between 1.3 and 1.5 $\log_{10}$, which is thus on a similar scale to that observed for experiments led with non-superheated steam (Table 3). Repeating the procedure 20 times led to no significant change in the standard deviation of the measurements, and 90% of the measurements fell within an interval that was twofold higher than the standard deviation. As previously systematic extreme microbial population's values were found for all the treatment durations (Table 3). In the case of the superheated steam treatment surface temperature is less repeatable than previously for the constant 96 °C surface temperature treatment (Table 1). It was checked that extreme population values do not correspond to differences in surface temperature and cannot explained by microbial experimental errors. The dispersion of the results and the non-correlation between the decimal reduction of the microbial population and the measured surface

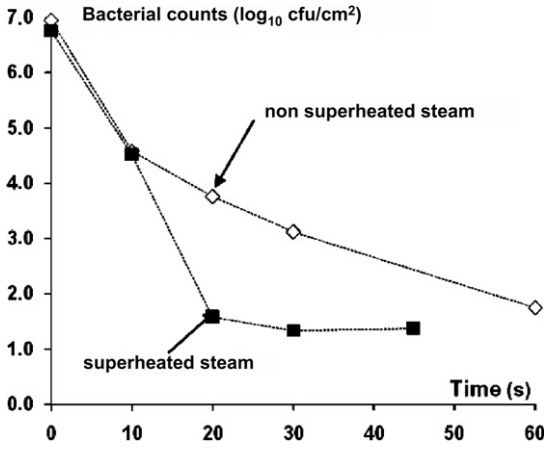

Fig. 4. Comparison of the effect of the superheated (black squares) and non-superheated (white diamonds) steam treatments on the inactivation of *Listeria innocua* (CLIP 20595) at the surface of poultry skin. Experimental points result from the averaging of the values obtained during the 10 repetitions (20 repetitions for the 30 s treatment). These values are highly variable which would leads to an overlapping of the standard deviation bars (Tables 2 and 3). Variance of results are analyzed by ANOVA in Table 5 to determine if the difference between the two cases: non-superheated or superheated steam are significant.

Table 3
Average, maximum and minimum *Listeria innocua* (CLIP 20595) populations measured at different treatment times for the superheated steam treatment; standard deviations on the experimentals values

Time (s)	Mean pop.	Max pop.	Min pop.	Std
0	6.8	7.0	6.1	0.2
10	4.5	6.0	2.3	1.4
20	1.6	3.9	0.5	1.3
30	1.3	3.5	0.0	1.5
45	1.4	3.6	0.0	1.4

Table 4
Average and maximum temperatures reached after 45 s of superheated steam treatment for the 10 experimental repetitions.

Average T (°C)	Maximum temperature	Decimal reduction
186	240	3.8
186	225	6.1
170	221	5.6
176	227	6.1
186	236	4.2
174	226	6.4
169	197	3.2
164	195	4.6
158	185	6.9
179	223	6.9

Microbial population measured at the end of each experiment.

temperature are given as an illustration in Table 4 for the 45 s thermal treatment.

The average microbial reduction obtained using either superheated or non-superheated steam was identical during the first 10 s of treatment. After this first 10 s, the superheated steam treatment leads to a far greater reduction in the bacterial population at the chicken skin surface. Difference between the two curves strongly diminishes after 30s of treatment. These average results should not hide the fact that microbial results are highly variable which would led to an overlapping of the standard deviations bars (values given in Tables 2 and 3) around the two curves if they were represented in Fig. 4. This would be the case even for the 20 and 30 s treatments. Thus, the variance of the relative reduction of the population ($\log_{10}(N/N_0)$ where N_0 is the initial population) was analyzed to determine if the average population reductions presented in Fig. 4 are significant or not (ANOVA, Table 5). Result proves that the average differences between the superheated and non-superheated steam treatments are very significant for the 20 and 30 s durations and not significant in the other cases. Therefore, the use of superheated steam may prove useful in industrial settings for treatment durations in between 10 and 30 s. This procedure can give highly efficient surface decontamination without cooking the meat protected under the carcass skin, and a process patent has thus been filed (N°. 05 53451). However, very-high-temperature superheated steam still cannot prevent the 'tailing' effect observed in previous studies on heat decontamination techniques applied to food surfaces. There are cases where

Table 5
Results of the analysis of the variance (ANOVA) performed on the reduction of the initial population ($\log_{10}(N/N_0)$) to characterize the difference of inactivation between the non-superheated and superheated steam treatments

Time (s)	Non-superheated		Superheated		ANOVA	
	Mean	Variance	Mean	Variance	F	P
10	2.35	1.19	2.25	2.02	0.033	0.8577
20	3.18	1.80	5.19	1.33	12.75	0.0020[a]
30	3.82	2.25	5.43	2.18	11.59	0.0016[a]
45	Assessed	Assessed	5.39	1.85	1.58	0.2241
	4.69	1.04				
60	5.24	1.42				

[a] Difference is very significant.

sub-populations of microorganisms can resist surface temperatures in excess of 220–240 °C (Table 4). This heat-resistance is not systematic, since decontamination can be in excess of 6 $\log_{10}$, or on contrary limited to 3.2–4.2 $\log_{10}$ for similar heat conditions (Table 4).

Preliminary experiments have appeared to show that decontamination efficiency may be correlated with the thickness of the skin sample. Therefore, we systematically

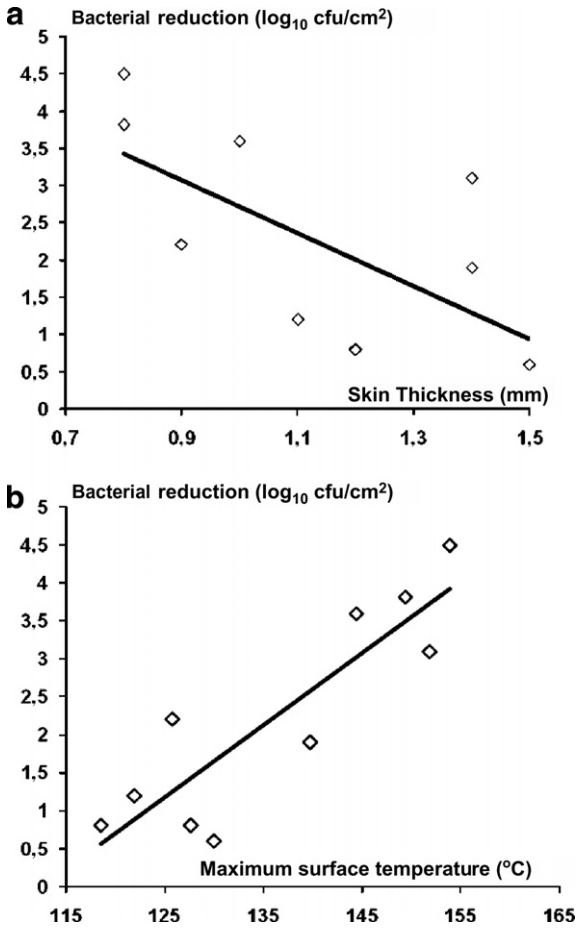

Fig. 5. Relationship between the reduction of the *Listeria innocua* population during the first 10 s of the superheated steam treatment and (a) the thickness of the poultry skin and (b) the maximum temperature measured at the surface of the sample.

measured skin thickness of the upper chicken leg on every sample before superheated steam treatment. The results showed that the average skin thickness of 49 samples was 1.1 mm, and that all the chicken samples presented a skin thickness falling within a ±0.4 mm bracket around the average thickness (i.e., between 0.7 and 1.5 mm), except for one outlier sample measured as 1.9 mm. We observed that during the first 10 s of treatment, decontamination was faster on thinner chicken skins (Fig. 5a). This effect was directly related to the peak skin surface temperature reached during the treatment (Fig. 5b). These correlations were not reproduced under longer treatment periods. However, the lowest decontamination value (only 2.0 $\log_{10}$) for a 30s superheated steam treatment was obtained in the experiment led on the thickest chicken skin (i.e., the 1.9 mm-thick skin sample). This lower decontamination value was also associated to a low surface temperature. However other experiments with similar low surface temperature on 1.0 mm skin thickness were not associated with low decontamination values. Therefore, it would appear that at treatment periods in excess of 10 s, the surface-inoculated bacteria on thicker skins show an increased resistance that is not necessarily directly related to surface temperature.

4. Discussion

The average *L. innocua* population reductions obtained in this study using non-superheated steam are similar to those reported on chicken skin by McCann and Sheridan et al. (2006) working with different microorganisms and slightly lower temperatures at the product surface. Using superheated steam made it possible to reach surface temperatures of 240 °C, which to the best of our knowledge marks the highest temperatures ever achieved on chicken skin surfaces during decontamination trials. The results demonstrate that even at these temperatures, certain *L. innocua* are still able to survive for over 45 s.

These results raise questions about the microbial inactivation treatments currently accepted by food industry. The results of Gaze et al. (1989) prove that a 6 $\log_{10}$ reduction in the *Listeria* population inoculated in homogenates of chicken and beef steak can be obtained in 2 min at 70 °C which will lead to an equivalent treatment of 5 s at 80 °C. Gaze et al. (1989) clearly recommend not extrapolating their results for temperature higher than 80 °C. However much shorter treatment times should be obtained in present temperature conditions (less than a second at 98 °C, when Gaze et al.'s results are extrapolated). The major difference with present study is the nature of the food product itself. In Gaze's work food products were pre-cooked (autoclaved for 5 min) and macerated with water while in present study a raw and fresh piece of product is directly subjected to a jet of steam. Industrials shall now be aware that currently accepted inactivation treatments are far to be enough on raw pieces of meat subjected to ambiances of hot air or steam.

In present work, the curve for average microbial inactivation over time follows the same pattern observed on raw products subjected to air jets or steam jets under conditions where the surface temperature remains below 100 °C. This suggests that although bacterial heat resistance varies according to experimental conditions and to type of microorganism, the inactivation pattern, characterized by a rapid decrease in pathogen numbers followed by a 'tailing' period, appears to be a general phenomenon for all microorganisms inoculated on the solid surfaces of Teflon® or foodstuffs whether subjected: to air jet treatments, to classical steam treatments or to superheated steam jet treatments (Gaze et al., 2006; McCann and McGovern et al., 2006; McCann and Sheridan et al., 2006; Valdramidis et al., 2006, in press). Working with a very high temperature at the chicken skin surface shortens the first rapid decline of microbial population achieving strong microbial reduction after 20 s of treatment.

Previous studies had underlined that heat decontamination techniques applied to food surfaces displayed strong heat resistance and poor repeatability. The repetitions that we systematically led in this study confirmed this phenomenon. But the painstaking controls over the steam jet, surface temperature measurements and computer-modeled simulations (Kondjoyan and Portanguen, in press) prove that this effect cannot be explained by poorly controlled heat treatment or local temperature variability within the products themselves. In this study, there was only one case where we were able to correlate the inactivation efficiency with the skin thickness: the 10 s superheated steam treatment. In all the other cases, the results did not lead to correlations. This can be explained by heat treatment simulations based on previously developed heat transfer models (Kondjoyan and Rouaud, 2006; Kondjoyan and McCann, 2006; Kondjoyan and Portanguen, in press). These simulations prove that temperature gradients exist in the chicken skin during the first 10–12 s of treatments while the whole skin remains at the same temperature afterwards, regardless of the skin thickness within the 1–2 mm bracket. One potential explanation of the strong heat resistance of surface inoculated microorganisms is that the microorganism is in fact somehow 'biologically protected' by product structure or composition. When chicken skin is heated, the fats melt rapidly and could protect the microorganism from inactivation. The conditions under which the fats fuse and mix with condensate inputs from the steam jet could generate different microenvironments around the microorganisms, which would explain the repeatability variations observed in consecutive experiments. However, this would only partially explain the results for chicken and does not explain similar tailing period results reported for meat pieces or potatoes (Gaze et al., 2006; McCann and McGovern et al., 2006; McCann and Sheridan et al., 2006) and even less so the results observed on abiotic media such as Teflon® (Valdramidis et al., in press).

During the Bugdeath project hot air was proved to be less efficient than steam for surface decontamination (James and Evans, 2006). This effect was explained by an

increasing thermal resistance of bacteria due to product surface desiccation. This explanation was comforted by the very sharp decrease of water activity predicted by transfer calculations (Kondjoyan and Rouaud, 2006; Kondjoyan and McCann, 2006; McCann and McGovern et al., 2006). The relative humidity of superheated steam diminishes as the superheat temperature increases. This phenomenon was put forward by Spicher et al. (2002) to explain their results on the less efficiency of superheated steam compared to saturated steam in decontaminating microbe spores. In the present study the superheated steam temperature, and thus the steam dehumidification levels, are much greater than during Spicher's experiments. Thus under these "dry conditions" dehumidified steam can loose part of its inactivation efficiency. However, results prove that even dry superheated steam treatments remain efficient to inactivate microorganisms in vegetative state.

Surface desiccation cannot explain the very important microbial resistance observed during saturated steam treatment. In this case the hypotheses put forward by McCann and Sheridan et al. (2006) to conclude their article, i.e., resistance induced either by microorganism adhesion onto solid media or by the heat treatment itself (heat shock proteins), remain the most plausible at the present time.

The average inactivation levels measured on superheated steam-treated *L. innocua* can be described using Eq. (1) and parameters in Table 6. However average experimental kinetics can never be fitted perfectly by (1) for all the treatment times (Fig. 6a and b). Moreover relation (1) does not rely on any backstage microbiological consideration. Thus the nine more basic inactivation models included in the GInaFIT software (Geeraerd et al., 2005) were tested to describe present results. Only three of them were able to fit correctly the observed inactivation patterns while leading to small residues (Table 7). Moreover the Geeraerd shoulder-tail's model leads to negative value for the shoulder parameter which is not coherent from a microbiologic point of view. Results calculated by the two remaining coherent models: the "Weibull-tail" model (Albert and Mafart, 2005) and the "biphasic-shoulder" model (Geeraerd et al., 2005) are compared to measurements in Fig. 6.

The Weibull plus tail model correspond to the following mathematical relation:

$$N = (N_0 - N_{res})10^{\frac{-pt}{delta}} + N_{res} \quad (2)$$

where N is the microbial population at time t (in seconds), N_0 and N_{res} the initial and the residual microbial population respectively. p and delta are parameters of the model given in Table 8. In present situation the "Weibull-tail" model requires a value of the average residual population less than the minimum averaging population measured.

Table 6
Estimates of the parameters R, B and A in relation (1) used to describe the reduction of the microbial population

Treatment/parameters	A	B	R	MSSE	RMSSE
96 °C Treatment	1.55	5.39	0.96	0.086	0.294
Superheated steam	0.42	6.34	0.94	0.170	0.413

MSSE is the mean sum of the square error between the results calculated by models and the experimental values. RMSSE is the root mean square of the square error.

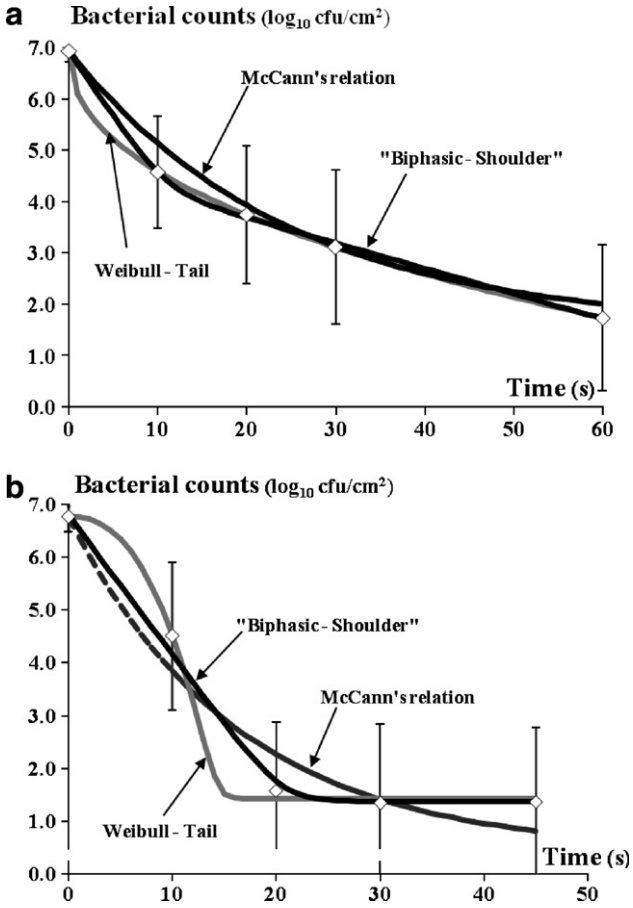

Fig. 6. Description of the inactivation of *Listeria innocua* (CLIP 20595) by relation (1) and by the two basic inactivation models included in the GInaFIT software which agree the best with experimental results in the two cases: (a) 96 °C treatment and (b) the superheated steam treatment.

In the "biphasic-shoulder" model the shoulder length is not used (shoulder length equals to 0) and the relation reduces to

$$\log(N) = \log(N_0(f e^{-k1\max t} + (1-f)e^{-k2\max t})) \quad (3)$$

where N is the microbial population at time t (in seconds), N_0 the initial population and f, k_{1max} and k_{2max} are parameters given in Table 8.

Residues calculated in both cases on the average microbial populations are very small (Table 7) and thus each of the two models is plausible. Interpretation on models cannot goes further as: (1) calculations are biased in the case of the superheated steam treatment by the non-integration of the surface temperature variations in the models, (2) experimental standard deviation is so important that it is illusory to discriminate models using present experimental results.

Attempts have been made by us to model our microbial results introducing a stochastic response to heat treatment into an elementary predictive microbiology model. The kinetics of microbial reduction were represented by

Table 7
Mean sum of the square error (MSSE) between the results calculated by models and the experimental values, root mean square of the square error (RMSSE)

Treatment	Model	Deviation on average values			Deviation on all values		
		MSSE	RMSSE	$R - S$	MSSE	RMSSE	$R - S$
96 °C	*Geeraerd shoulder-tail*	*0.0024*	*0.0492*	*0.9998*	*1.8600*	*1.3600*	*0.3900*
96 °C	Weibull-tail	0.0002	0.0152	1.0000	1.8600	1.3600	0.3900
96 °C	Biphasic-shoulder	0.000	0.007	0.999	1.900	1.380	0.390
S. Steam	*Geeraerd shoulder-tail*	*0.0004*	*0.0192*	*1.0000*	*1.7800*	*1.3300*	*0.5500*
S. Steam	Weibull-tail	0.0343	0.1851	0.9986	1.7800	1.3400	0.5500
S. Steam	Biphasic-shoulder	0.032	0.178	0.993	1.850	1.360	0.540

The three chosen inactivation models are those included in the GInaFIT software which agree the best with experimental results in the two cases: 96 °C treatment and superheated steam treatment. The Geeraerd shoulder-tail's model leads to negative value for the shoulder parameter which is not coherent from a microbiologic point of view.

Table 8
Parameters of the Weibull-tail model (2) and of the biphasic model (3) which minimize the mean sum of the square error between the results calculated by models and the experimental values

Treatment	Weibull-tail model's parameters				Biphasic-shoulder model's parameters (shoulder length = 0)			
	$\log(N_0)$	$\log(N_{res})$	p	Delta	$\log(N_0)$	k_{max1}	k_{max2}	f
96 °C	6.93	0.87	0.46	1.69	6.93	0.57	0.11	0.99
S. Steam	6.76	1.31	1.26	5.24	7.13	0.64	0.05	1.00

first-order reduction laws (classical decimal reduction models), and stochastic variability was introduced on the model's decimal reduction parameter. This variability could be interpreted either as the selection of more or less resistant microbial populations during microbe adhesion, as a variability related to the genesis of heat shock proteins, or as a variability related to the effects of other as yet unidentified phenomena. The model was able to reproduce the inactivation pattern that is observed when experimental results are averaged out (rapid decrease in pathogen numbers followed by a 'tailing' period). But calculations lead to higher variation and standard deviation that those measured in the study reported here, which almost certainly reflects the fact that the decimal model is too minimalist to be able to describe experimentally observed phenomena. If this kind of approach is to be validated scientifically, it will require a hundred-odd repeats of each experiment, which in practical terms is a stumbling block since decontamination trials on microorganisms adhered to solid surfaces are particularly resource- and labor-intensive. Better predictive models cannot be developed until progress is made in our understanding of the mechanisms underlying the very high heat-resistance of solid–surface–adherent bacteria in gaz treatment. Understanding these mechanisms would make it possible to achieve workable predictions of heat-based surface decontamination as well as a better evaluation of the health risks involved in improperly cooking solid foodstuffs.

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