PREDICTIVE MODELS FOR THE SURVIVAL OF *CAMPYLOBACTER JEJUNI* INOCULATED IN CHICKEN MEAT

LUIS PABLO SANSALONE[1], JUAN MARTÍN OTEIZA[2,3], GABRIELA GIACOBONI[1] and LEDA GIANNUZZI[2]

[1]*Facultad de Veterinaria*
Universidad Nacional de La Plata
La Plata, Argentina

[2]*CIDCA, CONICET*
Facultad Ciencias Exactas
Universidad Nacional de La Plata
47 y 116, La Plata 1900, Argentina

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ABSTRACT

Campylobacter jejuni *is an emerging pathogen that is becoming more relevant due to the high percentage of isolations found in raw or undercooked poultry products. The objective of the present work was to analyze and to model mathematically the effect of storage temperature (+4 and −2C), potassium sorbate concentration (0, 500 and 1,000 ppm) and packaging in plastic films of different gas permeabilities (low and high gas permeability, polyethylene and EVA SARAN EVA) on the survival of* C. jejuni *inoculated into chicken meat. Different models (linear, Gompertz and Weibull equation) were applied to experimental data, obtaining the best fit with the Weibull model. Among analyzed factors, storage temperature (+4 and −2C) was the most effective factor to inhibit growth of* C. jejuni *in chicken meat that reduce microbial counts between 3 and 4 logarithmic cycles. However, at −2C, the inhibitory effect was more successful. Potassium sorbate and packaging in different permeability films did not show inhibitory effect on* C. jejuni.

PRACTICAL APPLICATIONS

The results of this study suggest that potassium sorbate and packaging films did not show inhibitory effect on *Campylobacter jejuni* inoculated in

[3] Corresponding author. TEL: +54-221-4254853; FAX: +54-221-4254853; EMAIL: jmoteiza@cidca.org.ar

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chicken meat. Storage temperature (+4 and −2C) is the most important factor in controlling the growth of *C. jejuni* in chicken meat.

INTRODUCTION

In humans, *Campylobacter* spp. causes campylobacteriosis, a gastrointestinal tract infection (Lindqvist *et al.* 2001; Adak *et al.* 2002; Samuel *et al.* 2004; Nauta and Havelaar 2008) characterized by severe diarrhea, fever, abdominal pains, nausea and vomiting that usually lasts for 5 to 7 days (Gallardo *et al.* 2006). The principal species responsible for the majority of infections is *Campylobacter jejuni* (Tortora *et al.* 2002). The symptoms of the infection are normally more serious in children, elderly people and persons with underlying health problems (Carneiro *et al.* 2008).

Foods, especially of animal origin, are the common source of *Campylobacters* spp, because a part of the intestinal flora of several animals are found in those foods (WHO 2002; Hussain *et al.* 2007; Little *et al.* 2008). Contaminated water supplies (Hirsh 1999b; Kemp *et al.* 2005), salad vegetables (Evans *et al.* 2003; Kärenlampi and Hänninen 2004; Chai *et al.* 2007), grapes (Neimann *et al.* 2003), contaminations by domestic animals (Hirsh 1999a; Hussain *et al.* 2007), milk or milk products (Michaud *et al.* 2004) that have not been pasteurized and meats, especially chicken, have also been shown to harbor these organisms (Doyle and Shoeni 1986; Giacoboni *et al.* 1999; NPHS 2006, Humphrey *et al.* 2007; Sallam 2007).

Poultry flocks are frequently colonized with *C. jejuni* without apparent symptoms (Shane 2000). Risk assessment analyses have identified handling and consumption of poultry meat as one of the most important sources of human campylobacteriosis (Evans *et al.* 2003; Potter *et al.* 2003; Friedman *et al.* 2004).

In several countries, *Campylobacter* species is essentially a foodborne pathogen and has recently overtaken *Salmonella* spp. (Park 2002).

Much of the world's poultry production is contaminated with campylobacters and this is reflected in the high isolation rate for these pathogens reported for poultry products sold in major supermarket outlets. For example, in the U.S.A., 69% of chickens bought from a local supermarket were found to be contaminated with *C. jejuni* and levels of contamination may vary between 10^2 and 10^5 cfu per carcass (Jacobs-Reitsma 2000).

Potassium sorbate is known by its inhibiting action on microbial growth (Robach and Ivey 1978; Robach and Sofos 1982; Zamora and Zaritzky 1987). Thus, it has been used to prolong storage life of several products, even of chicken meat.

Many food products are packaged in special atmospheric environments and stored at low temperatures to reduce or prevent the growth of some microorganisms, and to extend the shelf life of the product.

Microbial inhibition also depends on the type of packaging film used (Zamora and Zaritzky 1987). Lee *et al.* (1999) did not detect differences in *C. jejuni* survival when the microorganisms were inoculated on chicken skin and packed with different films.

It is well known that some pathogens and spoilage microorganisms are inhibited by temperatures below 4C; thus, control temperature is one of the strategies used to preserve fresh products. Although, the fact that the *C. jejuni* does not multiply very effectively below 4C in most foods, refrigeration does not prevent it from causing foodborne illnesses

In recent years, great interest has been taken in the storage of foods and aging of processed foodstuffs at a subfreezing temperature (near −2C) (Tanaka *et al.* 1999). Currently, the effect of subfreezing temperature on the inactivation of microorganisms is not clear.

The combined effect of films with different gas permeabilities and low storage temperatures on *C. jejuni* survival has not been yet explored.

Predictive microbiology is a combination of mathematical modeling and bacterial growth/inactivation responses to several factors such as temperature, pH and water activity (A_w) (Membre *et al.* 2005). Predictive microbiology is a basic tool for estimating microbial behavior in foods during processing and storage. This kind of models allows to predict safety and shelf life of food products under different environmental conditions, and to evaluate risk assessment for human health.

The objectives of this research were: to analyze and to model simultaneously the effect of storage temperature (+4 and −2C), potassium sorbate addition (0, 500 and 1,000 ppm) and gas permeability of films on the survival of *C. jejuni* inoculated onto chicken meat.

MATERIAL AND METHODS

Bacterial Strains and Inoculum Preparation

The *C. jejuni* (CJ107) strain used in this study was isolated from eviscerated fresh chicken (Faculty of Veterinary Science, La Plata University). It was characterized phenotypically by their ability to hydrolyze hippurate and indoxil acetate, production of catalase and sensitivity to nalidixic acid and cephalothin. It was biotypified as Lior II scheme. CJ107 strain was grown on Brucella agar (Merck KGaA, Darmstadt, Germany) containing 5% (v/v) defibrinated sheep blood. Plates were incubated for 24–48 h at 42C under a

microaerophilic atmosphere achieved by using *Campylobacter* gas-generating kits (Oxoid, BR56, Basingstoke, England) in conjugation with their catalyst containing anaerobic jars (Oxoid). A culture under stationary phase and with an abundant growth was obtained; these conditions allowed an important bacterial recuperation, as well.

Cell suspension was prepared in 10 mL of physiological saline by scraping the colonies grown on the surface of Brucella agar with 5% sheep blood, and was maintained at (Merck KGaA) 4C until analysis.

Preparation of Chicken Meat Samples

Chickens were provided by a commercial poultry abattoir. Muscles predominantly from the breast region had a postmortem period of 24 h at 4C. All samples were stored at 4C for less than 4 h until analysis. To reduce microbial counts, chicken breasts were placed in a sterile container with 10% chlorine solution for 30 min. Then, they were washed with sterile distilled water to eliminate remaining chlorine; samples were drained off on a sterile lattice. Finally, chicken breasts were sprayed with 70% ethanol and flamed for few seconds. Samples were aseptically cut and chopped with an industrial machine, previously sterilized (Rowenta Universo, Francia), to get minced meat. A biological biohazard cabinet was used to obtain the ground chicken meat.

Randomly selected samples were tested for the presence of *Campylobacter* species. Also, the initial pH and the A_w of the minced meat were determined by an electrode (model 50215, Hach, Loveland, CO) on a pH meter (model EC30, Hach) and by an AQUALAB equipment (model CX-2, Ann Arbor, MI).

Inoculation of Chicken Meat Samples with *C. jejuni*

Samples of 500 g of chicken minced meat were aseptically placed into a sterile stomacher bag with 40 mL of sterile 0.1% (w/v) peptone water (Merck KGaA) and then inoculated with 5 mL with the overnight culture of *C. jejuni* CJ107 in order to achieve inoculum levels of 6.00 log cfu/g. Samples were homogenized for 2 min in a stomacher (Stomacher 400 Lab Blender, Seward Medical, London, UK). After inoculation, the stomacher bag was kept at room temperature (approximately 20 $\pm$ 1C) for 30 min to allow attachment of the bacteria.

Inoculated samples were divided in three lots. Just prepared solutions of 5 and 10% potassium sorbate were added to two of the lots, to get final concentrations of 500 and 1,000 ppm in the meat samples. The third lot, without potassium sorbate addition (0 ppm), was used as the control. Samples were again homogenized for 2 min in the stomacher to obtain an adequate distribution of the additive. Then, each lot was divided in aliquots of 50 g, and

packaged with two different films: EVA SARAN EVA (ESE) (O_2 and CO_2 permeabilities of 50 and 105 $cm^2/m^2/24$ h/1 atm/23C, respectively) and polyethylene (O_2 and CO_2 permeabilities of 5,000 and 0 $cm^2/m^2/24$ h/1 atm/23C, respectively). ESE samples were packaged under vacuum using a Minidual equipment model MW 4980 (Schcolnik SAIC, Buenos Aires, Argentina) with a single chamber at 4.5 mm Hg and heat sealing. Samples packaged in polyethylene were sealed without vacuum. Packaged samples were stored in controlled temperature chambers, under different temperatures (+4 and -2 ± 0.1C) for 20 days.

Microbiological Examination

At regular intervals (48 h) postinoculation, a single package of chicken minced meat was removed from each storage temperature. Subsamples of 10 g were taken at random from each packaged sample and aseptically weighed, put into a sterile stomacher bag with 90 mL of sterile 0.1% (w/v) peptone water and homogenized for 1 min in the stomacher. Serial dilutions (1:10) were performed with sterile 0.1% peptone water, followed by plating of selected dilutions in duplicate on Brucella agar containing 5% (v/v) defibrinated sheep blood. All bacterial plates were incubated for 24–48 h at 42C under a microaerophilic atmosphere achieved by using *Campylobacter* gas-generating kits (Oxoid, BR56) in conjugation with their catalyst containing anaerobic jars (Oxoid).

Typical colonies of *C. jejuni* were counted after incubation and log cfu/g values were calculated. Presumptive identification was performed by the appearance of the colonies, microscope examination for curved to s-shaped or spiral form with typical motility, Gram stain and positive reaction to catalase and oxidase test.

C. jejuni species were confirmed phenotypically by their ability to hydrolyze hippurate and indoxil acetate, sensitivity to nalidixic acid and cephalothin. Simultaneously, at the end of the storage period, counts of the natural microbiota of chicken meat were performed in Brucella agar (48 h, 37C) as culture medium. A total of 20 colonies randomly selected were characterized through the following assays: Gram stain, oxidase and catalase test, nitrate reduction test, growth in Cetrimide agar (Merck) after incubation for 48 h at 37C and in MacConkey agar (Merck) after incubation for 20 h at 37C, and gelatin liquefaction (Mac Faddin 1979).

Mathematical Models

Three mathematical models were used in this study.

One of these was the linear model:

$$\mathrm{Log}\,(S) = -t/\mathrm{D} \tag{1}$$

where S is the survival fraction (N/N_0) at treatment time t and D is the decimal reduction time (mathematically, the inverse of the inactivation curve slope).

The modified Gompertz equation is given by the following expression (Linton *et al.* 1996):

$$\mathrm{Log}\,(N/\mathrm{N}_0) = d[\exp(-\exp(fg)) - \exp(-\exp(-\mathrm{f}(t-g)))] \tag{2}$$

where N/N_0 has the same meaning as in Eq. (1), t is the time and the three parameter estimates (d, f and g) represent the different regions of the survival curve: f is the initial shoulder that can be present in the curve, g is the maximum death rate and d is the overall change in the survivor number. Data were fitted to the modified version of the Gompertz equation.

Peleg and Cole (1998) reinterpreted the microbial survival curve as the cumulative form of the temporal distribution of lethal events. According to this concept, each individual bacterium in the population dies or is inactivated at a specific time. There is a spectrum of treatment resistances in the population and the shape of the survival curve will be determined by shape distribution having different distribution parameters. Survival data were fitted to the cumulative form of the frequency Weibull distribution of resistance (Peleg and Cole 1998).

$$\mathrm{Log}\,S(t) = \log N/N_0 = -bt^n \tag{3}$$

where b and n are constants. The n factor describes the shape of the survival curves so that, when $n < 1$, the survival curve is concave upward (it forms tails); when $n > 1$, the survival curves is concave downward (it forms shoulders); and when $n = 1$, the survival curve is a straight line on a log scale.

The values b and n were then used to plot the resistance-frequency curve using the following equation:

$$d\Phi/dt_\mathrm{c} = b\cdot n\cdot t_\mathrm{c}^{(n-1)}\exp\left(-b\cdot t_\mathrm{c}^{\,n}\right) \tag{4}$$

and if its member's resistance expressed in terms of their t_{ci} can be considered as having a continuous where t_c is a measure of organism resistance or sensitivity, Φ is the fraction of organisms sharing any given t_c, and $d\Phi/dt_c$ is a Weibull distribution corresponding t_c. Thus, if the Weibull distribution is considered as an appropriate representative mathematical model of the population, its parameters b and n can be estimated by fitting Eq. (3) to the survival data. Once calculated, these values of b and n can be used to plot the

sensitivities/resistance-frequency curve using Eq. (4), and to calculate the distributions mode: t_{cm}, mean: $\overline{t}_c$, variance: $\sigma^2 t_c$ and coefficient of skewness: ν_1 from the following equations (Peleg and Cole 1998)

$$t_{cm} = [n-1]^{1/n} \tag{5}$$

$$\overline{t}_c = \{\Gamma[(n+1)/n]\}\,b^{1/n} \tag{6}$$

$$\sigma^2 t_c = \left\{\Gamma[(n+2)/n] - \Gamma[(n+1)n]^2\right\} b^{2/n} \tag{7}$$

$$\nu_1 = \mu_3 / \mu_2^{3/2} \tag{8}$$

where Γ is the gamma function,

$$\mu_3 = \Gamma(1+2/n)/b^{3/2} \text{ and } \mu_2 = \Gamma(1+2/n)/b^{2/n} \tag{9}$$

The distribution mode t_{cm} represents the treatment time at which the majority of population dies or inactivates. The mean $\overline{t}_c$ corresponds to the inactivation time on average with its variance, $\sigma^2 t_c$. The coefficient of skewness $\sigma^2 t_{c1}$ represents the skew of the distribution. It is related to the most resistant members of the population.

Model Comparison

The following three criteria were used to compare the linear, Gompertz and Weibull models:

Mean Square Error. The goodness of fit was evaluated with the mean square error (MSE) (Ross 1996), which represents a measure of accuracy computed by squaring the individual error of each item in the data and then finding the average or mean value of the sum of these squares (Rodrigo *et al.* 2003). The smaller the MSE values, the better is the fit of the model to the data (Neter *et al.* 1996; Chen 2007).

$$\text{MSE} = \sum (\text{predicted} - \text{observed})\,2/n - \text{p} \tag{10}$$

where the letter n stands for the number of observations and p for the number of parameters to be estimated.

Coefficient of Determination (R^2) Values. The higher value, the better is the adequacy of the model to describe data (Neter *et al.* 1996).

Accuracy Factor. The accuracy factor (A_f) was proposed by Ross (1996) to evaluate the performance of predictive models. This factor provides a measure of the average difference between observed and predicted values and is defined as:

$$A_f = 10 \sum (\log(\text{predicted}/\text{observed}))/n \quad (11)$$

The larger the A_f values, the less accurate is the average estimate, while a value of 1 indicates that the model produces a perfect fit to these data.

Experimental Design and Statistical Analysis

A factorial design was used: three concentrations of potassium sorbate added (0, 500 and 1,000 ppm), two films with different gas permeabilities (ESE and polyethylene) and two storage temperatures (+4 and −2C). Therefore, a total of 12 experimental conditions were tested. All tests were carried out in duplicate.

Data fit obtained from different models was analyzed by means of Systat software (Systat, Evanston, IL). This software calculates the set of parameters with the lowest residual sum of squares and their 95% confidence interval. Besides, it provides for each data fit the sum of squares, the degree of freedom (df) and the mean square due to the regression and the residual variation.

RESULTS AND DISCUSSION

Microbiological Characterization

Chicken meat samples had a pH between 5.70 and 5.90 and a A_w of 0.98, both conditions that highly favor microbial growth. Aseptically washed uninoculated samples did not show any traces of intrinsic campylobacters (data not shown).

At the end of the storage period, the natural microbiota of chicken meat grown in Brucella agar was analyzed with different tests: Gram stain, oxidase and catalase test, nitrate reduction test, growing in MacConkey and Cetrimide agar and gelatin liquefaction. These tests allowed to determine that *Pseudomonas* spp. were the dominant genera in chicken meat, which could compete for nutrients affecting *C. jejuni* growth (data not shown). Blankenship and Craven

(1982) reported that *Pseudomonas* spp. become a predominant spoilage flora when chicken meat is packaged in air and stored at low temperature, whereas *Lactobacillus* spp. are more prevalent when chicken meat is packaged in carbon dioxide and stored at low temperature. They also reported that *C. jejuni* can survive with the spoilage flora in both packaging atmospheres examined, and this fact may cause difficulties in determining the causative microorganism involved in a foodborne disease outbreak.

Survival Curves of *C. jejuni* in Chicken Meat

Initial bacterial population (N_0, cfu/mL) of *C. jejuni* inoculated in chicken meat ranged between 4.8×10^5 and 3.4×10^6 cfu/g.

Figure 1a–f shows survival curves of *C. jejuni* inoculated onto chicken meat stored at +4 and −2C, treated with 0, 500 and 1,000 ppm of potassium sorbate and packaged in different permeability films.

In all cases, at the end of the storage period, between 15 and 20 days, the number of *C. jejuni* decreased to 3 or 4 log (N/N_0).

The addition of 500 and 1,000 ppm of potassium sorbate did not show inhibitory effect with respect to the control samples and the use of different permeability film did not increase the inactivation of *C. jejuni*. Analysis of variance (ANOVA) in this study was carried out to determine the statistical significance among the different treatments. ANOVA did not show significant differences in campylobacter survival among potassium sorbate at 500 or 1,000 ppm and for films of different gas permeabilities. However, the effect of storage temperature (+4C and −2C) on the survival count was significant ($P < 0.05$).

It is well known that *Campylobacter* remains viable and multiplies in bile at 37C and survives better in feces, in milk at 4C than in material held at 25C. The maximum periods of viability of *Campylobacter* spp. at 4C were 3 weeks in feces, 4 weeks in water and 5 weeks in urine. Freezing reduces the cell populations of *Campylobacter* spp. in contaminated poultry, but even after freezing to −20C, low levels of *Campylobacter* can be recovered (Jacobs-Reitsma 2000).

The different patterns of inactivation curves were analyzed in terms of three mathematical models: linear, Gompertz and underlying distribution of resistances and its statistical parameters from Weibull model. To compare the goodness of fit of the three models, the MSE, R^2 and A_f values were evaluated.

Among the three mathematical models tested, the best fit (lowest MSE, greatest coefficients of determination (R^2) and an overall A_f values) was obtained when the experimental data were adjusted to the Weibull distribution function.

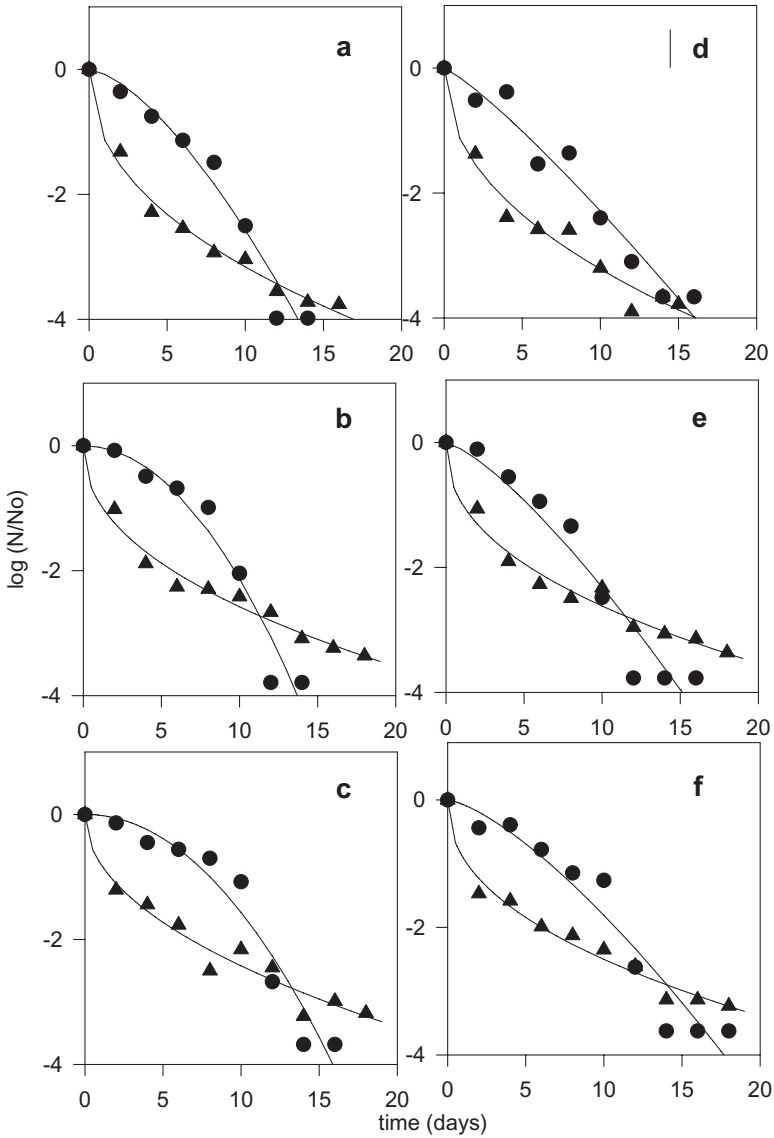

FIG. 1. FITTING OF WEIBULL TYPE DISTRIBUTION MODEL TO SURVIVAL CURVES OF *CAMPYLOBACTER JEJUNI* INOCULATED ONTO CHICKEN MEAT PACKAGED IN EVA SARAN EVA (a, b AND c), AND POLYETHYLENE (d, e AND f); TREATED WITH: 0 ppm POTASSIUM SORBATE (a AND d), 500 ppm POTASSIUM SORBATE (b AND e) AND 1,000 ppm POTASSIUM SORBATE (c AND f). SYMBOLS: ●: +4C AND ▲: −2C

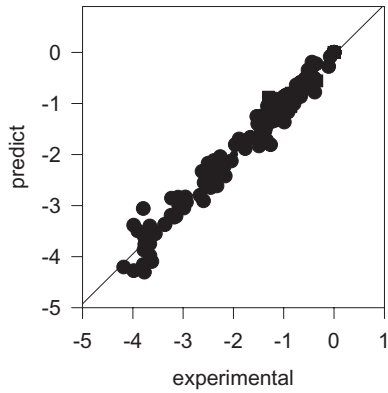

FIG. 2. CORRELATION BETWEEN EXPERIMENTAL AND PREDICTED DATA USING WEIBULL DISTRIBUTION MODEL

For all the data, the MSE values were: 0.0456, 0.1516 and 0.0334; R^2 were: 0.8817, 0.879 and 0.9780; and A_f values were 1.17, 1.16 and 1.05 for linear, modified Gompertz and Weibull models, respectively. The df were 149. The Weibull model was appropriate for the description of the survival of *C. jejuni* cells exposed at different storage conditions. Figure 1 exhibits the survival curves generated with the Weibull model and the experimental data. Characteristic sigmoid shapes of Weibull type distribution appeared. Appropriate residual distribution was also verified (data not shown). A good correlation between experimental and predicted data was obtained with Weibull model (Fig. 2).

Since it produced a reasonably good fit to all the survival curves, the Weibull model was further analyzed to determine the effect of stored temperature, addition of potassium sorbate and the use of different gas permeabilities films on the b and n values of Weibull model.

Table 1 shows b and n parameters estimated by fitting model (Eq. 3).

Analysis of n values indicated that temperature significantly affected the shape of survival curves. Survival curves of *C. jejuni* inoculated onto samples packaged in different films showed downward concavity ($n > 1$) when stored at +4C, while those stored at −2C showed upward concavity ($n < 1$). In this case, a distribution with a strong right skew clearly related to a marked upward concavity was observed. It can be argued that weak members of the population were destroyed at a fast rate, leaving behind a little fraction of higher resistance survivors (Peleg *et al.* 1997) and likely indicating a mixed population with different resistances to the freeze/thawing process. For samples stored at 4C ($n > 1$), a possible explanation of these curves shapes could be that the

TABLE 1.
WEIBULL TYPE DISTRIBUTION PARAMETERS OF *CAMPYLOBACTER JEJUNI* IN CHICKEN MEAT WITH AND WITHOUT POTASSIUM SORBATE, PACKAGED WITH FILMS OF DIFFERENT GAS PERMEABILITYIES (POLYETHYLENE AND EVA SARAN EVA, ESE) AND STORED AT +4 AND –2C

T(C)	Treatment	b	n	t_{cm}	$\bar{t}_c$	$\sigma^2 t_c$	ν_1
Polyethylene							
4	Control	0.08 ± 0.03	1.51 ± 0.09	4.48	4.78	12.75	1.56
4	500	0.02 ± 0.02	1.98 ± 0.33	10.08	5.86	22.10	6.79
4	1,000	0.01 ± 0.01	2.05 ± 0.32	13.74	7.60	37.65	11.18
–2	Control	1.13 ± 0.09	0.44 ± 0.03	–	1.34	–0.07	1.45
–2	500	0.90 ± 0.09	0.45 ± 0.04	–	2.20	–0.14	0.73
–2	1,000	0.78 ± 0.12	0.48 ± 0.06	–	2.74	–0.05	0.52
ESE							
4	Control	0.15 ± 0.06	1.17 ± 0.22	0.21	5.06	11.78	0.67
4	500	0.11 ± 0.06	1.32 ± 0.22	0.422	5.05	12.96	0.96
4	1,000	0.07 ± 0.04	1.39 ± 0.02	0.51	6.29	20.87	1.18
–2	Control	1.12 ± 0.13	0.45 ± 0.05	–	1.35	–0.05	1.40
–2	500	0.96 ± 0.12	0.43 ± 0.04	–	1.97	–0.21	0.88
–2	1,000	0.90 ± 0.09	0.44 ± 0.04	–	2.25	–0.21	0.73

t_{cm}, mode (day); $\bar{t}_c$, mean (day); $\sigma^2 t_c$ variance (day^2); ν_1, coefficient of skewness.

more sensitive members were weakened, leaving a large fraction of more resistant members which were affected in much lesser extent (Peleg 2000).

In all the assays, as storage temperature decreased, *b* parameter increased evidencing once again a temperature effect on microorganism survival.

To obtain a better explanation about the influence of the storage temperature, type of permeability film and potassium sorbate addition on *C. jejuni* inactivation, the *b* and *n* values were used to generate resistance to the treatments in terms of frequency distribution plots (Fig. 3).

Table 1 also resumes the underlying distribution parameters: mode, mean, variance and coefficient of skewness for each frequency distribution calculated according to Eqs. (5) to (8). For samples stored at 4C, frequency distribution shapes indicated substantial overall spread of the data with correspondent mean values greater than those stored at –2C. The addition of 500 and 1,000 ppm of potassium sorbate in samples stored at 4C distribution was more skewed to the right than control samples, showing that an important fraction of the *C. jejuni* can survive under this treatment.

For samples with and without potassium sorbate, packaged in different gas permeability films and stored at –2C, the frequency distribution calculated according to Eq. (4) does not have a peak, as seen in Fig. 3c,d. This fact could indicate that the majority of microorganisms were inactivated in a short time after the exposure to –2C.

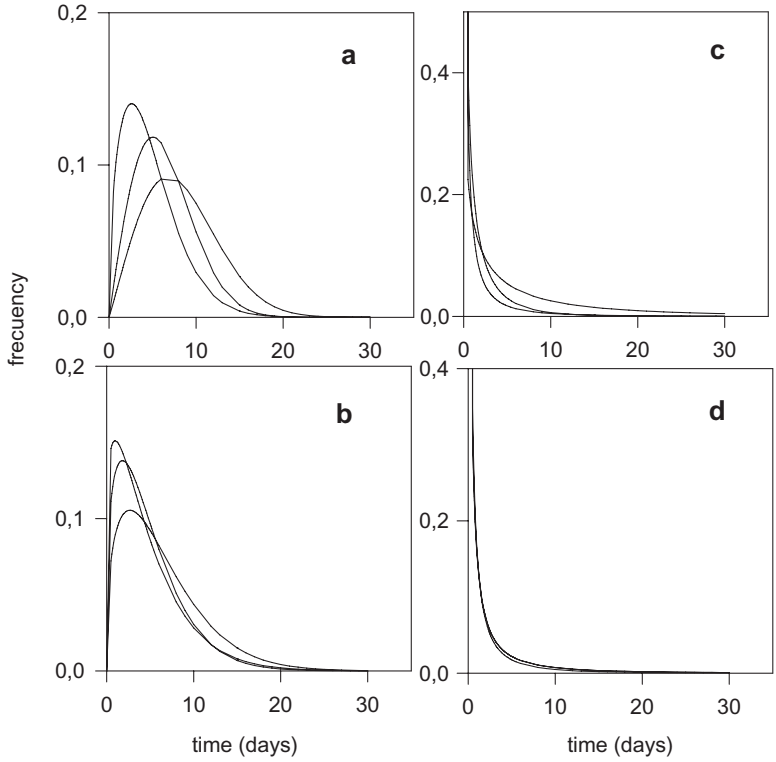

FIG. 3. DISTRIBUTION OF THE RESISTANCES CORRESPONDING TO *CAMPYLOBACTER JEJUNI* SURVIVAL INOCULATED ONTO CHICKEN MEAT PACKAGED IN EVA SARAN EVA (b AND d) AND POLYETHYLENE (a AND c), STORED AT +4C (a AND b) AND −2C (c AND d). POTASSIUM SORBATE ADDED: (—) 0 ppm, (---) 500, AND (·····) 1,000 ppm

In the case of *C. jejuni* inoculated on samples of meat chicken with addition of 1,000 ppm of potassium sorbate and packaged in polyethylene and ESE and stored at 4C, it took much more time to weaken the majority of the population. The distribution mean values $\overline{t}_c$ increased 1.60- and 1.24-fold with respect to control system in polyethylene and ESE packaged, respectively, and had much more spread evidenced by the high variance values. The Weibull type distribution model evidenced differences between stored conditions concluding that the temperature stored at −2C was the most effective condition to inactivated *C. jejuni* in meat chicken showing the lowest inactivation time on average, $\overline{t}_c$.

In relation to the survival of *C. jejuni* at low temperatures, Hazeleger *et al.* (1998) observed differences in survival profiles at low temperature of different strains but does not explain the reason for these observation.

In campylobacters, the adaptive mechanisms underlying the responses to cold stress have not been examined in any detail, although this is of considerable interest, given the involvement of chilled foods in infection. *Campylobacters* show marked changes in the fatty acid composition of chilled cells compared with those held at higher temperatures and there are differences in protein expression between cultures held at either 4 or 20C (Humphrey *et al.* 2007). There is still much to learn about the ability of *C. jejuni* to survive low temperature.

While many bacteria produce characteristic cold shock proteins, and these are associated with their ability to replicate at temperatures below the optimum growth temperature, an analysis of the *C. jejuni* genome sequence indicates that campylobacters do not produce this type of cold shock protein (Park 2002).

C. jejuni is metabolically active at temperatures far below its minimal growth temperature and is able to perform respiration and generate adenosine triphosphate at temperatures as low as 4C. At even lower temperatures, viability is rapidly lost. While several factors, including ice nucleation and dehydration, have been implicated in the freeze-induced injury of bacterial cells, more recently, oxidative stress has been shown to contribute to the freeze–thaw-induced killing of campylobacters (Stead and Park 2000).

Other authors working with different *C. jejuni* strains analyzed the effect of several preservatives (sodium ascorbate, sodium chloride, potassium nitrate, sodium nitrite and polyphosphates) on microorganism survival and found different effects depending on the strain used (Uradzinski and Szteyn 1993). Özdemir *et al.* (2006) study the antimicrobial effects of acidified sodium chloride and trisodium phosphate in chicken breast skin inoculated with *C. jejuni* stored at 4C during 5 days. These authors observed that the effect of all antimicrobials increased during the storage period, and the bacterial counts reached undetectable levels ($<1.0 \times 10^2$ cfu/g) on day 5 of storage.

The effect of packaging film was not significant on *C. jejuni* survival. These results agreed with those of Lee *et al.* (1999), who analyzed the effect of polyethylene on samples of chicken skin inoculated with *C. jejuni* packaged under vacuum and stored at 4C. These authors did not find an inhibitory effect of permeability film on *C. jejuni* survival. This fact could be attributed to the microaerofila nature of *C. jejuni* that is not inhibiting under these conditions.

The results of this study indicated that potassium sorbate alone or combined with films of different gas permeabilities did not have additional inhibitory effect on *C. jejuni*, which the temperature effect produced. However, the storage temperature (+4C and −2C) was effective in inhibiting the microorganism in chicken meat.

Low temperature is widely applied in the food chain, and it is vital that more become known about how this pathogen responds to these conditions

with regard to survival and subsequent behavior, as this has the greatest overall relevance to food safety.

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