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Modelling the low temperature growth boundaries of *Salmonella* Enteritidis in raw and pasteurized egg yolk, egg white and liquid whole egg: Influence of the initial concentration

Silvia Guillén [a], Lara Domínguez [a], Pilar Mañas [a], Ignacio Álvarez [a], Elena Carrasco [b], Guillermo Cebrián [a,*]

[a] *Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Instituto Agroalimentario de Aragón - IA2 - (Universidad de Zaragoza-CITA), Zaragoza, Spain*

[b] *Departamento de Bromatología y Tecnología de los Alimentos, Campus de Excelencia Internacional Agroalimentario CeiA3, UIC ENZOEM, Universidad de Córdoba, Campus de Rabanales, 14071 Córdoba, Spain*

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ABSTRACT

Salmonella is the most frequently reported cause of foodborne outbreaks with known origin in Europe, with eggs and egg products standing out as the most frequent food source (when it was known). The growth and survival of *Salmonella* in eggs and egg products have been extensively studied and, recently, it has been reported that factors such as the initial concentration and thermal history of the egg product can also influence its growth capability. Therefore, the objective of this study was to define the boundary zones of the growth/no growth domain of *Salmonella* Enteritidis (4 strains) as a function of temperature (low temperature boundary) and the initial concentration in different egg products. A series of polynomial logistic regression equations were successfully adjusted, allowing the study of these factors and their interaction on the probability of growth of *S.* Enteritidis in these products. Results obtained indicate that the minimum growth temperatures of *Salmonella* Enteritidis are higher in egg white (9.5–18.3 °C) than in egg yolk (7.1–7.8 °C) or liquid whole egg (7.2–7.9 °C). Results also demonstrate that in raw liquid whole egg and raw and pasteurized egg white, the minimum growth temperature of *Salmonella* Enteritidis does depend on the initial concentration. Similarly, the previous thermal history of the egg product only influenced the minimum growth temperature in some of them. On the other hand, large differences in the minimum growth temperatures among strains were observed in some products (up to approx. 6 °C in egg white). Finally, it should be noted that none of the strains grew at 5 °C under any of the conditions assayed. Therefore, storage of egg products (particularly whole liquid egg and egg yolk) below this temperature might be regarded/proposed as a good management approach. Our experimental approach has allowed us to provide a more accurate prediction of *S.* Enteritidis minimum growth temperatures in egg products by taking into account additional factors (initial concentration and thermal history) while also providing a quantification of the intra-specie variability. This would be of high relevance for improving the safety of egg products.

1. Introduction

Salmonella is the most frequently reported cause of foodborne outbreaks with known origin in Europe, and in 2021, 27% of human cases of salmonellosis associated with a known food source in this region were linked to eggs and egg products consumption (EFSA, 2022). Among more than 2500 *Salmonella* serovars, Enteritidis is the most commonly isolated serovar from eggs and egg products. So, the growth and survival of this serovar in eggs and egg products have been extensively studied (De Vylder et al., 2013; Gantois et al., 2009). In this respect, it is well known that there are large differences in the growth capacity of *Salmonella* depending on the egg fraction (yolk *vs* white), growth temperature and other factors. One of the most important environmental factors influencing bacterial population growth is the temperature. *Salmonella* is capable to grow in a wide range of temperatures, ranging from 5 to 47 °C with an optimum of 35 to 42 °C (D'Aoust, 1989). In egg products the minimum growth temperature of *S.* Enteritidis is around 6–8 °C (Kang et al., 2021; Kim et al., 2018; Whiting and Buchanan,

* Corresponding author.
E-mail address: guiceb@unizar.es (G. Cebrián).

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1997). The egg fraction also influences the growth rates and yields of *Salmonella*, being much higher in egg yolk than in egg white (Guillén et al., 2021; Kim et al., 2018; Messens et al., 2004; Moon et al., 2016). This difference has been attributed to the particular composition and physico-chemical characteristic of the latter, which is particularly efficient in inhibiting/slowing microbial growth (Baron et al., 1997, 2016). Recently, it has been reported that other factors such as the initial cell concentration and thermal history also influence the growth capability of *Salmonella* in egg and egg products (Guillén et al., 2020a, 2021; Kang et al., 2021). These phenomena seem to be related to the effect of heat treatments on the antimicrobial activity of some egg white proteins (such as ovotransferrin and lysozyme) and on the dose-dependent ability of *Salmonella* cells to uptake iron (Baron et al., 2016; Lechevalier et al., 2017; Scholz and Greenberg, 2015).

Kinetic growth models have been developed to predict the growth of *Salmonella* in egg products in order to establish the optimal temperature and time for their preservation and distribution (Kang et al., 2021; Kim et al., 2018; Li et al., 2017; Singh et al., 2011). In addition to these predictive kinetic models, probabilistic growth/no growth (G/NG) interface models have been developed as they provide information on microbial growth limits –which is also required for defining the range of applicability of growth models-. Several growth/no growth models have been developed for *Salmonella* (Basti and Razavilar, 2004; Koutsoumanis et al., 2004; Lanciotti et al., 2001; Pin et al., 2011), including factors with major influence in growth such as temperature, pH and a_w. However, there are still many other factors potentially affecting/defining *Salmonella* growth boundaries, such as the influence of the initial number of cells, the thermal history of the food product, or the physiological state of the cells that have only been scarcely, if ever, investigated. In fact, these factors were already pointed out as future research needs to improve current *Salmonella* growth models (Carrasco et al., 2012). In addition, it should be noted that most of the available G/NG models for *Salmonella* were developed using data obtained in laboratory media. Although, in general, these models provide consistent predictions, might not take into account significant factors for microbial growth, such as food structure, competition/interaction with other microorganisms or the physiological state of microbial cells, among others. Therefore, works like validation of models in real foods systems or the development of new G/NG models derived from data obtained in food products, seem to be extremely useful to obtain more reliable and accurate predictions.

Recent studies have demonstrated the influence of the initial cell number and the thermal history on the growth fitness of *Salmonella* in egg white and liquid whole egg (Guillén et al., 2020a, 2021; Kang et al., 2021). Therefore, the objective of this study was to determine the influence of these factors and their interaction on the minimum growth temperature of *Salmonella* Enteritidis in whole liquid egg and its fractions. Intra-serovar variability was also investigated through the inclusion in the study of four *S.* Enteritidis strains. A series of polynomial logistic regression equations were built (including one for each combination of strain and egg fraction) in order to study the effect of these factors and their interactions on the probability of growth of *S.* Enteritidis in these products.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Four strains belonging to *Salmonella enterica* serovar Enteritidis were used in this study, (STCC 4300, STCC 4155, STCC 4396 and STCC 7160), that were supplied by the Spanish Type Culture Collection. The source of the strains (for which it is known) is included in Supplementary Material (Table S1). Strains were maintained at −80 °C in cryovials for long-term preservation. Cultures were grown in tryptic soy broth (Oxoid, Basingstoke, UK) supplemented with 0.6% *w/v* yeast extract (Oxoid, TSB-YE) in 96 wells microtiter plates and incubated at 37 °C as described in Guillén et al. (2020b).

2.2. Growth media

Growth experiments were carried out in raw liquid whole egg, egg white and yolk obtained from medium-sized raw eggs (53–63 g) and in commercial pasteurized liquid whole egg, egg white and egg yolk, purchased from a local supermarket.

2.3. Experimental design

The effect of initial concentration, incubation temperature and egg fraction on the growth/no growth of *S.* Enteritidis were determined. The selection was based on the limiting levels of the mentioned factors to the G/NG domain of *S.* Enteritidis, reported on previous studies in raw and pasteurized egg and eggs products (Guillén et al., 2021; Kang et al., 2021; Kim et al., 2018; Sakha and Fujikawa, 2012). Data were collected at 6, 6.5, 7, 7.5, 8 and 8.5 °C for whole liquid egg and egg yolk. Preliminary results showed that no growth was detected at 5 °C or below. For egg white the following temperatures were studied: 7, 8, 9, 10, 12, 15, 17.5 and 20 °C. The initial concentrations were 10^2, 10^3, 10^4, 10^5 and 10^6 CFU/mL. The experimental conditions are summarized in Table 1. The number of conditions per strain was 164, divided between the different egg products: raw and pasteurized liquid whole egg, raw and pasteurized egg yolk and raw and pasteurized egg white. The number of replicates per condition was 24.

2.4. Inoculation procedure

Appropriate dilutions of stationary phase cultures in buffered peptone water (Oxoid, BPW) were used to inoculate each well at the different initial inoculum concentration for each of the four Enteritidis strains. 24 replicate microtiter wells per condition and strain were filled with 297 μL of the different egg products, and inoculated with 3 μL of the appropriate bacterial dilution, achieving the desired initial concentration. After inoculation, the microtiter plates were sealed with a polyester impermeable film (VWR International, Leuven, Belgium) and placed in a

Table 1
Experimental conditions and levels of temperature, initial concentration and egg product considered for growth/no growth model for *Salmonella* Enteritidis strains.

Egg product	Temperature (°C)	Initial concentration (CFU/mL)
Raw whole egg	6.0	10^2, 10^3, 10^4, 10^5 and 10^6
Raw whole egg	6.5	10^2, 10^3, 10^4, 10^5 and 10^6
Raw whole egg	7.0	10^2, 10^3, 10^4, 10^5 and 10^6
Raw whole egg	7.5	10^2, 10^3, 10^4, 10^5 and 10^6
Raw whole egg	8.0	10^2, 10^3, 10^4, 10^5 and 10^6
Raw whole egg	8.5	10^2, 10^3, 10^4, 10^5 and 10^6
Pasteurized whole egg Egg yolk	6.0	10^2, 10^4 and 10^6
Pasteurized whole egg Egg yolk	6.5	10^2, 10^4 and 10^6
Pasteurized whole egg Egg yolk	7.0	10^2, 10^4 and 10^6
Pasteurized whole egg Egg yolk	7.5	10^2, 10^4 and 10^6
Pasteurized whole egg Egg yolk	8.0	10^2, 10^4 and 10^6
Pasteurized whole egg Egg yolk	8.5	10^2, 10^4 and 10^6
Egg white	7.0	10^2, 10^3, 10^4, 10^5 and 10^6
Egg white	8.0	10^2, 10^3, 10^4, 10^5 and 10^6
Egg white	9.0	10^2, 10^3, 10^4, 10^5 and 10^6
Egg white	10.0	10^2, 10^3, 10^4, 10^5 and 10^6
Egg white	12.0	10^2, 10^3, 10^4, 10^5 and 10^6
Egg white	15.0	10^2, 10^3, 10^4, 10^5 and 10^6
Egg white	17.5	10^2, 10^3, 10^4, 10^5 and 10^6
Egg white	20.0	10^2, 10^3, 10^4, 10^5 and 10^6

FX Incubator (Zeulab, Zaragoza, Spain) at the corresponding temperatures. Temperature was recorded using a calibrated type K thermocouple temperature sensor (Almemo, Ahlborn, Germany), with an accuracy of ±0.2 °C, connected to a data logger (Data logger 710, Almemo Ahlborn, Germany).

2.5. Growth/no growth evaluation and data selection

The occurrence of growth was tested after 30 days of incubation in all conditions and products assayed. For egg white it was also tested after 15 days. The bacterial population in each well was determined by surface plating on Xylose Lysine Deoxycholate agar (Oxoid, XLD) and compared with the initial count. Preliminary assays were performed also using tryptic soy agar +0.6% *w/v* yeast extract (Oxoid, TSA-YE) as the recovery medium. Since no differences between the media (data not shown) were observed, the subsequent analyses were exclusively conducted on XLD to avoid potential interference of the egg microbiota. Growth was confirmed when a difference of >0.5 log CFU/mL with the initial concentration was detected. When growth was confirmed, it was registered as "1", and "0" if it was not. The classification criterion was carried out at a cut point of $p = 0.5$, being p = probability of growth. In parallel, data were examined in order to detect possible outliers (*i.e.* decrease of the probability of growth when environmental conditions are less severe, or *vice versa*). The procedure followed was that proposed by Gysemans et al. (2007); an outlier condition was considered an unusual change of >10% in the observed growth probability compared to the neighboring data point. This was tested by comparing neighboring data points in the temperature and concentration directions separately.

2.6. Development of growth/no growth models

A polynomial logistic regression equation was fitted to data. The equation used in this study was a second-order linear logistic regression model, as follows:

$$\text{logit}(p) = a_0 + a_1 \cdot T + a_2 \cdot D + a_3 \cdot T \cdot D + a_4 \cdot T^2 + a_5 \cdot D^2$$

where p is the probability of growth, logit (p) equals ln (p / (1 - p)), a_0 – a_5 are the coefficients, T is temperature (°C) and D is initial concentration (log CFU/mL).

This model was fitted in XLSTAT® software (version 2019.2.2, Addinsoft, Boston) by using the forward stepwise procedure for regression and applying Firth's correction (Wald criterion with a significance of $p = 0.05$ and $p = 0.015$ for stepwise entry and removal, respectively).

2.7. Evaluation of models performance

Once models were obtained, for each model, goodness of fit statistics and predictive performance indexes were calculated in XLSTAT® software. In accordance with Valero et al. (2009), the maximum rescaled R^2 statistic, Pearson residuals and the Receiver Operating Characteristic (ROC) curve were used as measures of goodness of fit of the models.

R^2-Nagelkerke is a modification of the Cox–Snell coefficient to assure that it can vary from 0 to 1; a better fit of the model entails higher values of R^2 (Nagelkerke, 1991). Pearson residuals were calculated as the Hosmer–Lemeshow statistic does not give information about the nature of the lack of fit. Pearson residuals measure the difference between observed and predicted events, taking into account the number of observations (Gysemans et al., 2007). The area under ROC curve, c, is a measure of discrimination, obtained from a plot sensitivity (the proportion of observed events that was correctly predicted), against the complement of specificity (the proportion of observed non-events that was correctly predicted to be non-events). The closer the c value to 1, the greater the discrimination (Agresti, 2002). In addition, to compare various models, the Akaike Information Criterion (AIC) was calculated, which estimates the out-of-sample prediction error, and thus, the relative quality of the statistical models for a given data set.

To better illustrate the fit of the developed models to the observed data, the predicted probabilities at 0.1, 0.5 and 0.9 were calculated holding temperature and the initial concentration constant in GraphPad PRISM® statistical software (GraphPad Prism version 8.00 for Windows, GraphPad Software, San Diego, California, USA).

2.8. Validation

The performance of the logistic models was evaluated comparing models predictions with growth data from independent experiments. Internal validation of the model was performed by selecting a data set within the interpolation region, comprised of 48 conditions for the different *S.* Enteritidis strains and egg products. The experimental conditions investigated are summarized in Table 2. The procedure followed for inoculation and evaluation was as described above. To evaluate the fit, goodness of the fit (R^2, *RMSE*) parameters and Pearson's correlation coefficients were calculated using GraphPad PRISM® statistical software.

3. Results

In this study, the low temperature growth boundary of four *S.* Enteritidis strains in different egg products was determined. First, the occurrence of growth at different temperatures was determined for 4 *S.* Enteritidis strains in 6 different egg products/fractions at different initial concentrations. In order to determine the experimental conditions to be assayed, some preliminary assays were performed using *S.* Enteritidis STCC 4300 at two concentrations (10^2 CFU/mL and 10^6 CFU/mL). Thus, for those media for which no effect of the starting concentration was observed (egg yolk and pasteurized whole egg) only 3 concentrations were assayed whereas for the rest (raw whole egg and egg white) 5 concentrations were tested. Fig. 1 shows, as a way of example, the results obtained for *S.* Enteritidis STCC 4300, in the 6 media tested when inoculated with 10^2 and 10^6 CFU/mL.

After assessing the growth in the different conditions assayed, a polynomial logistic equation was fitted to data as described above. Since a transition from no growth to growth occurred in a very narrow temperature interval (around 0.5 °C) (Fig. 1), leading to the generation of perfect (or almost perfect) separations in most of the study scenarios –making consequently the maximum likelihood estimate infinite, the Firth penalty term was added to the log-likelihood function (Firth, 1993). A total of 30 logistic regression models were obtained in raw and pasteurized liquid whole egg, egg white and egg yolk. Once the models for each strain were obtained, another model was developed for each egg product by fitting to all data corresponding to the four strains (Global model).

Table 3 shows the equations obtained for each strain and egg product and the goodness of the fit, including the R^2-Nagelkerke and the area under ROC curve (AUC) value. Hosmer- statistic should be interpreted

Table 2
Experimental conditions and levels of temperature, initial concentration and egg product considered for validation of growth/non-growth models for *Salmonella* Enteritidis strains.

Egg product	Temperature (°C)	Initial concentration (CFU/mL)
Raw and pasteurized whole egg	7.25	$10^{4.5}$
Raw and pasteurized whole egg	7.75	$10^{2.5}$
Raw and pasteurized egg white	15.0	$10^{4.5}$
Raw and pasteurized egg white	17.5	$10^{2.5}$
Raw and pasteurized egg yolk	7.25	$10^{4.5}$
Raw and pasteurized egg yolk	7.75	$10^{2.5}$

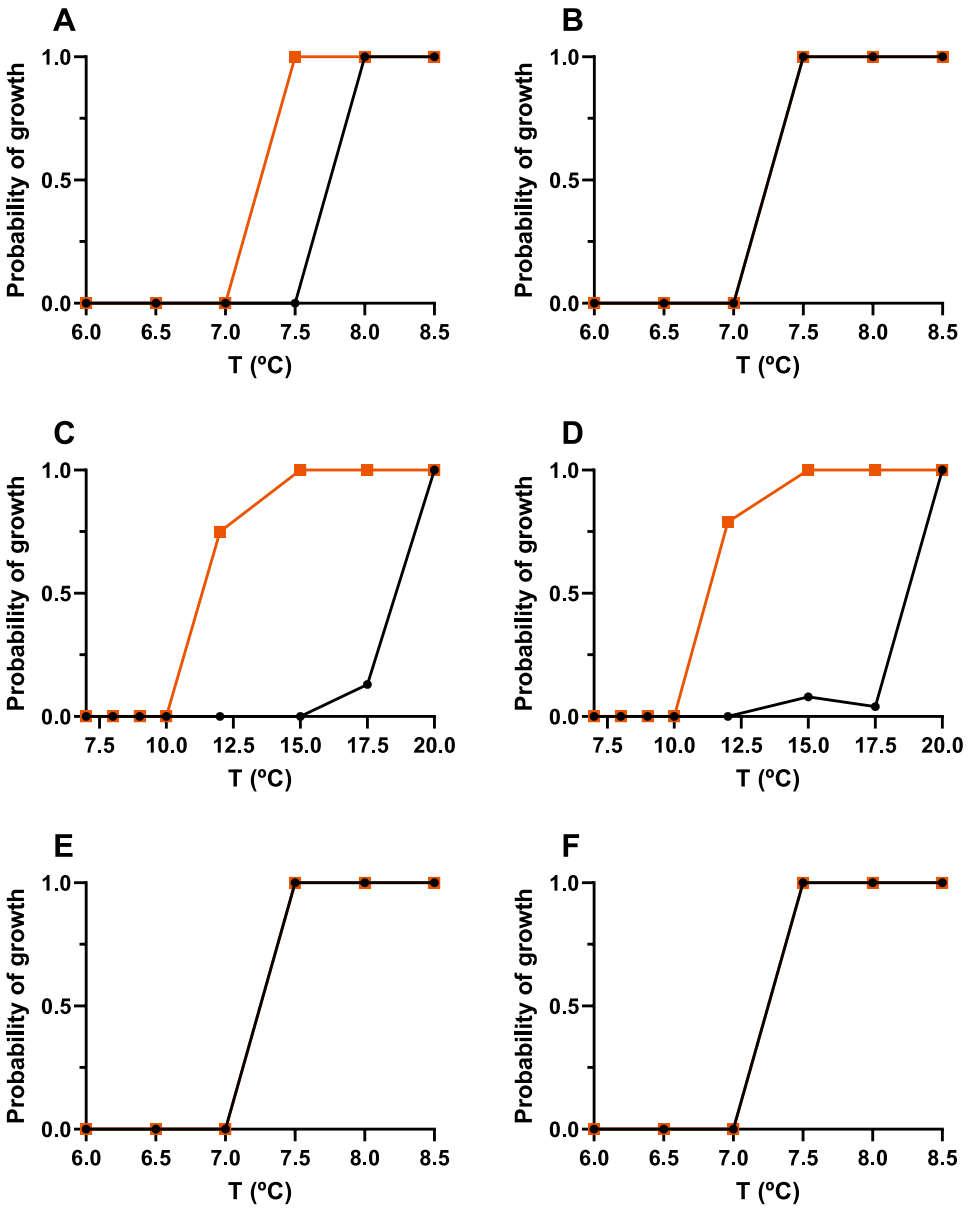

Fig. 1. Probability of growth of *Salmonella* Enteritidis STCC 4300 as a function of temperature, at the lowest initial concentration, 10^2 CFU/mL (black circles and line), and at the highest concentration tested, 10^6 CFU/mL (orange squares and line), and in the different egg products: raw liquid whole egg (A), pasteurized liquid whole egg (B), raw egg white (C), pasteurized egg white (D), raw egg yolk (E) and pasteurized egg yolk (F).

with caution as its value can be largely influenced by a single bad prediction, and therefore, in this study the Pearson residuals were calculated instead. These performance statistics indicate a reasonable goodness of fit of the models obtained, including the Global models (data from the 4 strains together). Additionally, the coefficients estimate and SE of the variables of the logistic regression models developed for the G/NG of *S.* Enteritidis strains in egg products, can be found in Table S2. In Table S2, only the coefficients of the significant variables ($p < 0.05$) are shown.

3.1. *Estimation of Salmonella Enteritidis minimum growth temperature in liquid whole egg*

As can be observed in Table 3, the R^2-Nagelkerke values of the models obtained for raw liquid whole egg varied between 0.933 and 0.972, AUC values between 0.994 and 0.998 and most of Pearson residuals were between −1 and 1, *i.e.*, the differences between the observed and predicted probabilities were small in relation to the number of observations. Thus, the percentage of values between −1 and 1 were 98.3, 97.2, 97.2 and 98.3% for 4155, 4300, 4396 and 7160 STCC respectively. As described above, the goodness of the fit of the global models was poorer, than the individual models, as was expected (R^2-Nagelkerke 0.850 *vs* 0.957 in average; concordance index 0.979 *vs* 0.997 in average; percentage of values of Pearson residual between −1 and 1 92.4 *vs* 97.8%, in average). Accordingly, in the classification table of observed *versus* predicted model conditions for *S.* Enteritidis strains (Table S3), it can be observed high percentage values of correct classification for all the models developed in raw liquid whole egg (ranging from 92.4% in the global model to 98.3% in the model of *S.* Enteritidis 7160 STCC strain).

Models describing the growth of *S.* Enteritidis strains in raw liquid whole egg were a function of the quadratic terms of initial concentration and temperature. The effect of these two variables (temperature and initial concentration) on the probability of growth of Enteritidis strains in raw liquid whole egg is depicted in Fig. 2. Fig. 2A shows the growth limits predicted for each strain, as a function of temperature and the

Table 3
Equations obtained by fitting the logistic regression model to the growth/non-growth data observed for *Salmonella* Enteritidis strains in the different egg products. The table also includes the global model equations, obtained after fitting together the data obtained for the four strains. The predictors of goodness of fit (R^2-Nagelkerke, the percentage of Pearson residuals values between −1 and 1 and the area under ROC curve) are also included. T is temperature (°C) and D is initial concentration (log CFU/mL).

Strain	Equation	R^2-Nagelkerke	Pearson residuals	AUC
Raw liquid whole egg				
4155	$\text{logit}(p) = -72.44 + 1.19 \cdot T^2 + 0.27 \cdot D^2$	0.967	98.3%	0.998
4300	$\text{logit}(p) = -63.78 + 1.07 \cdot T^2 + 0.19 \cdot D^2$	0.942	97.2%	0.994
4396	$\text{logit}(p) = -70.66 + 1.03 \cdot T^2 + 0.13 \cdot D^2$	0.933	97.2%	0.994
7160	$\text{logit}(p) = -79.88 + 1.31 \cdot T^2 + 0.17 \cdot D^2$	0.972	98.3%	0.998
Global model	$\text{logit}(p) = -26.67 + 0.42 \cdot T^2 + 0.08 \cdot D^2$	0.850	92.4%	0.979
Pasteurized liquid whole egg				
4155	$\text{logit}(p) = -52.34 + 0.97 \cdot T^2$	0.958	98.15%	0.993
4300	$\text{logit}(p) = -44.85 + 0.81 \cdot T^2$	0.923	94.91%	0.988
4396	$\text{logit}(p) = -46.027 + 0.71 \cdot T^2$	0.873	93.75%	0.979
7160	$\text{logit}(p) = -44.24 + 0.80 \cdot T^2$	0.920	94.44%	0.986
Global model	$\text{logit}(p) = -19.82 + 0.35 \cdot T^2$	0.820	88.02%	0.962
Raw egg white				
4155	$\text{logit}(p) = -3.81 - 0.34 \cdot T - 3.18 \cdot D + 0.29 \cdot T \cdot D + 0.02 \cdot T^2 + 0.12 \cdot D^2$	0.918	94.6%	0.991
4300	$\text{logit}(p) = 10.35 - 2.13 \cdot T - 4.89 \cdot D + 0.33 \cdot T \cdot D + 0.07 \cdot T^2 + 0.29 \cdot D^2$	0.892	95.5%	0.988
4396	$\text{logit}(p) = 0.56 - 3.41 \cdot T + 3.34 \cdot D + 0.09 \cdot T \cdot D + 0.16 \cdot T^2 - 0.34 \cdot D^2$	0.923	97.6%	0.993
7160	$\text{logit}(p) = 15.00 - -3.250 \cdot T - 4.77 \cdot D + 0.33 \cdot T \cdot D + 0.12 \cdot T^2 + 0.30 \cdot D^2$	0.909	96.7%	0.990
Global model	$\text{logit}(p) = -27.70 + 0.97 \cdot T + 3.89 \cdot D - 0.05 \cdot T \cdot D + 0.02 \cdot T^2 - 0.18 \cdot D^2$	0.874	95.0%	0.986
Pasteurized egg white				
4155	$\text{logit}(p) = 30.10 - 3.32 \cdot T - 16.31 \cdot D + 1.11 \cdot T \cdot D + 0.06 \cdot T^2 + 0.76 \cdot D^2$	0.951	98.09%	0.996
4300	$\text{logit}(p) = 18.91 - 2.40 \cdot T - 9.46 \cdot D + 0.37 \cdot T \cdot D + 0.08 \cdot T^2 - 0.81 \cdot D^2$	0.917	97.45%	0.991
4396	$\text{logit}(p) = 40.06 - 4.33 \cdot T - 16.53 \cdot D + 0.41 \cdot T \cdot D + 0.15 \cdot T^2 + 1.58 \cdot D^2$	0.965	99.55%	0.997
7160	$\text{logit}(p) = 67.77 - 5.01 \cdot T - 33.97 \cdot D + 0.72 \cdot T \cdot D + 0.16 \cdot T^2 - 3.43 \cdot D^2$	0.948	98.87%	0.996
Global model	$\text{logit}(p) = 0.50 - 0.79 \cdot T - 4.21 \cdot D + 0.17 \cdot T \cdot D + 0.04 \cdot T^2 - 0.43 \cdot D^2$	0.878	94.47%	0.987
Raw and pasteurized egg yolk				
4155	$\text{logit}(p) = -72.24 + 1.37 \cdot T^2$	0.997	100.0%	0.999
4300	$\text{logit}(p) = -72.24 + 1.37 \cdot T^2$	0.997	100.0%	0.999
4396	$\text{logit}(p) = -45.23 + 0.70 \cdot T^2$	0.873	92.4%	0.975
7160	$\text{logit}(p) = -42.39 + 0.76 \cdot T^2$	0.911	91.9%	0.981
Global model	$\text{logit}(p) = -20.48 + 0.36 \cdot T^2$	0.830	87.3%	0.959

initial concentration, by the logistic regression models obtained by setting the growth probability at 0.1 (10%) and Fig. 2B the growth limits calculated for the global model and the model obtained after calculating the coefficients average from the equations developed for each strain (average approach) from the equations developed for each strain and fixing the probability of growth at 0.1, 0.5 and 0.9 (10, 50 and 90%, respectively). An inverse relationship was obtained between the initial concentration and the minimum growth temperature; thus, the larger the inoculum size, the lower the minimum growth temperature. The highest minimum growth temperatures were obtained for *S.* Enteritidis 4396 STCC. In contrast, the lowest minimum growth temperatures, were obtained for strains 4155 and 4300 STCC, for which the influence of temperature and initial concentration was similar (Fig. 2A). Fig. 2B shows the different outcome resulting from using two approaches for estimating the minimum temperature growth boundaries for the *Salmonella* Enteritidis serovar set: the first one would be the result of the global model, and the second, by calculating the average of the minimum growth temperatures (for each probability) determined for the 4 strains individually (average approach). In Fig. 2B it can be observed that, as it would be expected, the minimum temperatures calculated for a probability of growth of 0.1 were lower when estimated with the first approach (global model) than when just calculating the average of the minimum temperatures determined for each strain and this probability level. The opposite was right when the minimum temperature required to ensure a probability of 0.9 was determined. Thus, results obtained indicate that the transition zone from $p = 0.1$ to $p = 0.9$ was much wider in the first approach (global model) than in the average approach.

Regarding pasteurized liquid whole egg, the goodness of the fit of the models developed was comparable to those developed for raw whole egg and also the goodness of the fit was better for the individual (strain) models than for the global model. However, in pasteurized liquid whole egg the models described the growth of *S.* Enteritidis strains as a function of only the quadratic term of temperature, that means that the growth limit temperatures were the same for all concentrations. The model that provided the best fit for the global model included both temperature and the quadratic temperature terms as significant parameters but, in order to simplify and facilitate comparisons, the temperature term was eliminated. Despite this change in the equation, the R^2-Nagelkerke value was the same, and the AIC value only increased by 2.78%. Fig. 2C shows the growth limit temperatures not dose-dependent, as pointed out above. In this case, two well-defined groups of strains were observed. On one hand, strains 4155, 4300 and 7160 STCC showed similar minimum growth temperatures, around 7.2 °C, whereas 4396 STCC strain required higher temperatures to be able to grow in pasteurized liquid whole egg, with more than 0.5 °C of difference, 7.8 °C.

If compared the minimum growth temperatures, it can be observed that in raw liquid whole egg this temperature was dose-dependent, as apposite to pasteurized liquid whole egg. However, the minimum growth temperatures, on average, at higher initial concentrations in raw and pasteurized liquid whole egg were the same, 7.4 °C (growth probability $p = 0.1$).

3.2. Estimation of Salmonella Enteritidis minimum growth temperature in egg white

Following the same methodology, a similar set of models defining the minimum growth temperature of *S.* Enteritidis in egg white (raw and pasteurized) was obtained. The goodness of the fit of these models was also good although slightly worse than those obtained for whole egg (Tables S2 and S3). As can be observed in the S2 and S3 Tables, the growth of *Salmonella* in egg white (both raw and pasteurized) was significantly influenced by inoculum size and temperature in all cases. However, since the equations did not include the same significant terms for all the strains, they were further homogenized, in this case by including all terms (linear, quadratic and interaction terms) to establish

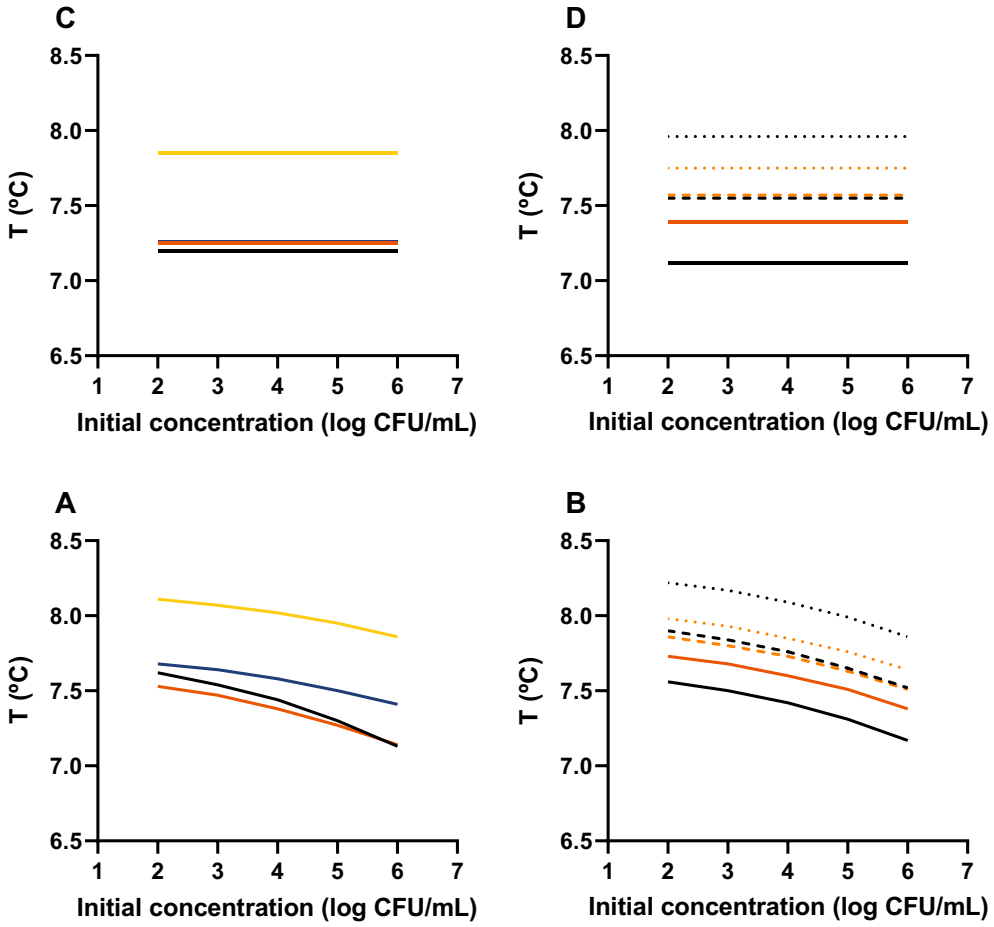

Fig. 2. Effect of temperature and initial concentration on the predicted probability of *Salmonella* Enteritidis strains in raw liquid whole egg (A-B) and in pasteurized liquid whole egg (C—D). Figs. A and C show the predicted growth limit temperatures determined with the logistic regression models developed for each of the different strains, *S.* Enteritidis 4155 STCC (black), *S.* Enteritidis 4300 STCC (orange), *S.* Enteritidis 4396 STCC (yellow) and *S.* Enteritidis 7160 STCC (blue), at a fixed probability of 0.1. Figs. B and D show the predicted growth limit temperatures estimated using the global model (black) and an average of the model parameters obtained for each strain individually (orange) for a growth probability of 0.1 (continuous line), 0.5 (dashed line) and 0.9 (dotted line).

comparisons (Table 3). The minimum growth temperatures (growth probability $p = 0.1$) calculated using these equations for each strain in raw and pasteurized egg white are shown in Fig. 3A and C. The growth limits calculated for the global model and the average model, fixing the growth probability at 0.1, 0.5 and 0.9, are shown in Fig. 3B and D for comparison purposes.

As can be observed, the minimum growth temperatures in egg white were higher, above 10 °C, compared to those in liquid whole egg. This difference could be attributed to several factors, including the higher content of antimicrobial proteins, increased viscosity and more alkaline pH in egg white, among other factors. It was also observed that the minimum growth temperatures in pasteurized egg white, although dose-dependent, were slightly lower than those in raw egg white, being the magnitude of this difference highly dependent on the strain studied.

These data are in agreement with those obtained by Kang et al. (2021), who reported that no growth of *Salmonella spp.* was observed at 5 °C in both raw and pasteurized egg white, as well as at 10 °C in raw egg white; however, slight growth in pasteurized egg white at 10 °C was observed. In addition, it should be noted that the influence of the initial concentration on the minimum growth temperatures in both raw and pasteurized egg white, were much higher than in raw liquid whole egg, with differences of >6.0 °C between the lowest and highest initial concentrations (Fig. 3). On the other hand, it is also evident that, conversely to pasteurized liquid whole egg, the minimum growth temperatures of *Salmonella* in pasteurized egg white was dependent on the initial concentration.

3.3. Estimation of Salmonella Enteritidis minimum growth temperature in egg yolk

Since in preliminary experiments no influence of the initial bacterial concentration or thermal history of the egg yolk on the minimum growth temperatures of *Salmonella* Enteritidis was found, a single model was constructed for this egg product. Again, good goodness of the fit values were obtained (Table 3 and S3). For the models developed in egg yolk the significant parameters were the linear temperature term for some strains, and for another, the quadratic temperature term, therefore for simplification purposes the quadratic temperature term was selected as it was for pasteurized whole liquid egg (Table 3). Despite this change in the equation, the R^2-Nagelkerke value did not change, and the AIC value only increased by 0.03% for strains STCC 4300 and 4155. In the case of the global model, the R^2-Nagelkerke changed from 0.839 to 0.830, and the AIC value increased by 4.2%.

The estimated minimum growth temperatures were similar to those obtained in pasteurized eggs for the different strains, with values between 7.1 and 7.8 °C (Fig. 4). This is consistent with data obtained in our previous work in which we observed that the growth rates of *Salmonella* were similar in raw and pasteurized yolk (Guillén et al., 2021) and also similar to those in pasteurized whole egg and even in raw whole egg inoculated at high concentrations.

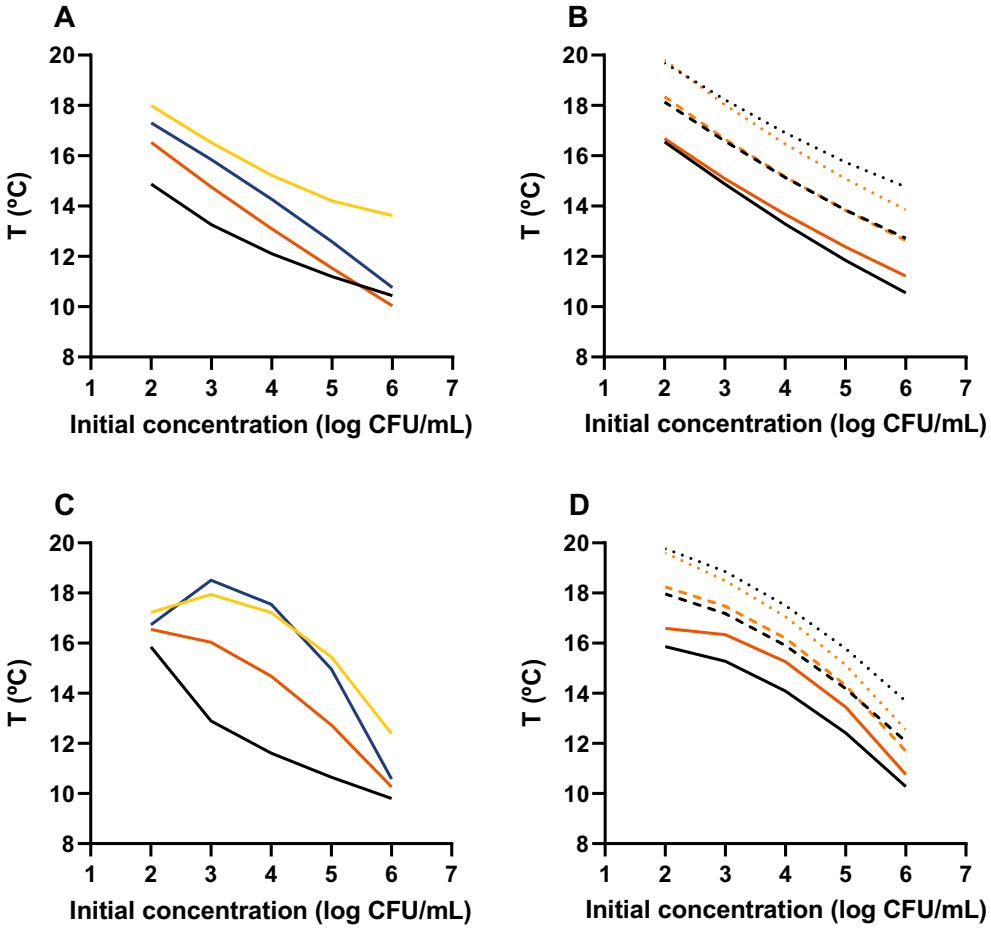

Fig. 3. Effect of temperature and initial concentration on the predicted probability of *Salmonella* Enteritidis strains in raw egg white (A-B) and in pasteurized egg white (C—D). Figs. A and C show the predicted growth limit temperature determined with the logistic regression models developed for each of the different strains, *S.* Enteritidis 4155 STCC (black), *S.* Enteritidis 4300 STCC (orange), *S.* Enteritidis 4396 STCC (yellow) and *S.* Enteritidis 7160 STCC (blue), at a fixed probability of 0.1. Figs. B and D show the predicted growth limit temperatures estimated using the global model (black) and an average of the model parameters obtained for each strain individually (orange) for a growth probability of 0.1 (continuous line), 0.5 (dashed line) and 0.9 (dotted line).

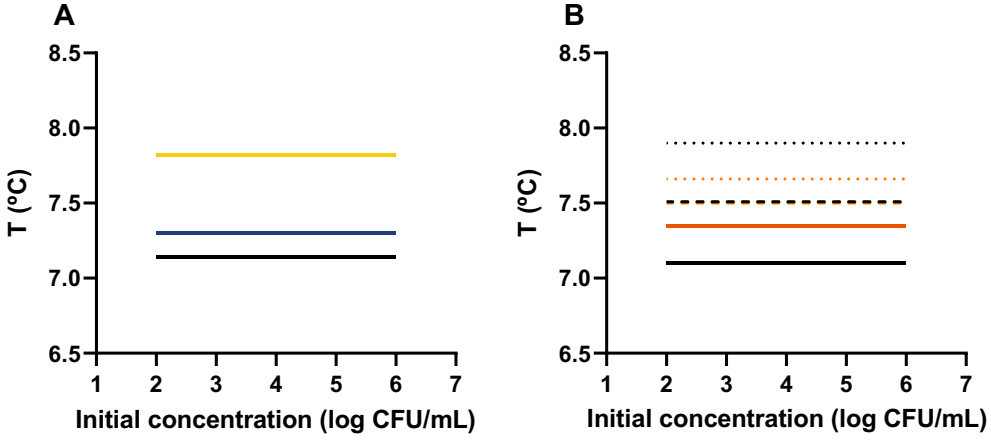

Fig. 4. Effect of temperature and initial concentration on the predicted probability of *Salmonella* Enteritidis strains in raw and pasteurized egg yolk (A-B). Fig. A shows the predicted growth limit temperature determined with the logistic regression models developed for each of the different strains, *S.* Enteritidis 4155 STCC (black), *S.* Enteritidis 4300 STCC (orange), *S.* Enteritidis 4396 STCC (yellow) and *S.* Enteritidis 7160 STCC (blue), at a fixed probability of 0.1. Figs. B show the predicted growth limit temperatures estimated using the global model (black) and an average of the model parameters obtained for each strain individually (orange) for a growth probability of 0.1 (continuous line), 0.5 (dashed line) and 0.9 (dotted line).

3.4. Validation

In order to evaluate the logistic regression models obtained, several boundary conditions were selected, in which not all replicates showed 100% growth or no growth ($0 < p$ observed <1) (Table 4).

Afterwards, a validation of the model was performed by selecting a

Table 4
Conditions under which boundary responses ($0 < p$ observed<1) were obtained for *Salmonella* Enteritidis strains and their corresponding predicted probability (p pred) for the developed models.

Strain	T (°C)	log (CFU/mL)	P_{obs}	P_{pred}	Strain	T (°C)	log (CFU/mL)	P_{obs}	P_{pred}
Raw liquid whole egg					**Pasteurized liquid whole egg**				
4155	7.5	3	0.08	0.05	4155	7.5	2	1.00	0.88
4155	7.5	4	0.21	0.25	4155	7.5	4	0.67	0.88
4155	7.5	5	0.79	0.79	4300	7.5	2	0.67	0.69
4300	7.5	3	0.29	0.16	4300	7.5	4	0.50	0.69
4300	7.5	4	0.33	0.42	4300	7.5	6	0.92	0.69
4300	7.5	5	0.79	0.80	4396	8.0	2	0.29	0.38
4396	8.0	4	0.13	0.08	4396	8.0	4	0.69	0.38
4396	8.0	5	0.21	0.21	4396	8.0	6	0.50	0.38
4396	8.0	6	0.50	0.52	7160	7.5	2	0.67	0.66
7160	7.5	5	0.13	0.09	7160	7.5	4	0.54	0.66
7160	7.5	6	0.38	0.40	7160	7.5	6	0.88	0.66
Raw egg white					**Pasteurized egg white**				
4155	17.5	2	0.33	0.53	4155	15.0	2	0.25	0.06
4155	15.0	3	0.63	0.42	4155	17.5	2	0.04	0.32
4155	15.0	4	0.75	0.85	4155	15.0	3	0.79	0.78
4155	15.0	5	0.88	0.98	4155	10.0	6	0.21	0.22
4155	12.0	6	0.79	0.66	4300	17.5	2	0.04	0.24
4300	17.5	2	0.13	0.24	4300	15.0	3	0.08	0.03
4300	12.0	4	0.13	0.03	4300	15.0	4	0.17	0.15
4300	15.0	4	0.42	0.56	4300	12.0	6	0.79	0.64
4300	12.0	5	0.38	0.17	4396	17.5	2	0.04	0.15
4300	15.0	5	0.92	0.95	4396	12.0	6	0.04	0.05
4300	12.0	6	0.75	0.68	7160	17.5	2	0.17	0.31
4396	15.0	4	0.04	0.07	7160	15.0	6	0.04	0.11
4396	12.0	5	0.13	0.01	7160	12.0		0.92	0.87
4396	15.0	5	0.21	0.27	**Raw and pasteurized egg yolk**				
4396	15.0	6	0.50	0.48	4396	8.0	2	0.46	0.46
7160	15.0	3	0.04	0.03	4396	8.0	4	0.46	0.46
7160	15.0	4	0.25	0.25	4396	8.0	6	0.46	0.46
7160	12.0	5	0.13	0.05	7160	7.5	2	0.50	0.51
7160	15.0	5	0.75	0.85	7160	7.5	4	0.54	0.51
7160	12.0	6	0.46	0.38	7160	7.5	6	0.50	0.51

data set within the interpolation region (Table 5). In Table 5, the performance of each model was evaluated, including the global models. In general terms, a good agreement was obtained between the observed values obtained and those predicted by the models, although with some exceptions. The best performances were observed for the whole egg, and the worst fits were for pasteurized egg white. Despite the differences

Table 5
Model validation conditions for *S.* Enteritidis strains in the different egg products and their corresponding observed probability (P_{obs}) and the different predicted probabilities for the developed models: models corresponding to each strain (P_{pred} strain) and model developed by fitting altogether the data corresponding to the four strains (P_{pred} global model).

Strain	T (°C)	log (CFU/mL)	P_{obs}	P_{pred} strain	P_{pred} global	Strain	T (°C)	log (CFU/mL)	P_{obs}	P_{pred} strain	P_{pred} global
Raw liquid whole egg						**Pasteurized liquid whole egg**					
4155	7.25	4.5	0.00	0.01	0.05	4155	7.25	4.5	0.00	0.18	0.17
	7.75	2.5	0.38	0.70	0.31		7.75	2.5	1.00	1.00	0.74
4300	7.25	4.5	0.00	0.03	0.06	4300	7.25	4.5	0.46	0.10	0.17
	7.75	2.5	0.29	0.87	0.32		7.75	2.5	0.96	0.98	0.74
4396	7.25	4.5	0.00	0.00	0.06	4396	7.25	4.5	0.00	0.00	0.17
	7.75	2.5	0.00	0.00	0.32		7.75	2.5	0.21	0.04	0.74
7160	7.25	4.5	0.00	0.00	0.06	7160	7.25	4.5	0.25	0.09	0.17
	7.75	2.5	0.29	0.39	0.32		7.75	2.5	0.88	0.98	0.74
Raw egg white						**Pasteurized egg white**					
4155	7.25	4.5	0.67	0.94	0.65	4155	7.25	4.5	0.88	1.00	0.47
	7.75	2.5	0.50	0.79	0.55		7.75	2.5	0.83	0.92	0.46
4300	7.25	4.5	0.96	0.83	0.65	4300	7.25	4.5	0.92	0.44	0.47
	7.75	2.5	0.54	0.49	0.55		7.75	2.5	0.67	0.30	0.46
4396	7.25	4.5	0.04	0.16	0.65	4396	7.25	4.5	0.13	0.00	0.47
	7.75	2.5	0.33	0.16	0.55		7.75	2.5	0.50	0.05	0.46
7160	7.25	4.5	0.50	0.56	0.65	7160	7.25	4.5	0.63	0.00	0.47
	7.75	2.5	0.63	0.32	0.55		7.75	2.5	0.71	0.02	0.46
Raw egg yolk						**Pasteurized egg yolk**					
4155	7.25	4.5	0.08	0.48	0.20	4155	7.25	4.5	0.08	0.48	0.20
	7.75	2.5	0.33	1.00	0.95		7.75	2.5	0.58	1.00	0.95
4300	7.25	4.5	0.04	0.48	0.20	4300	7.25	4.5	0.08	0.48	0.20
	7.75	2.5	1.00	1.00	0.95		7.75	2.5	0.75	1.00	0.95
4396	7.25	4.5	0.00	0.00	0.20	4396	7.25	4.5	0.00	0.00	0.20
	7.75	2.5	0.00	0.05	0.95		7.75	2.5	0.00	0.05	0.95
7160	7.25	4.5	0.25	0.06	0.20	7160	7.25	4.5	0.25	0.06	0.20
	7.75	2.5	0.92	0.95	0.95		7.75	2.5	0.67	0.95	0.95

between observed and predicted values (in most conditions), the predicted values were still within the transition zone. In any case, when analyzing these discrepancies, the complexity of the medium and the very narrow temperature range of the transition zone, <0.5 °C, should be taken into account.

4. Discussion

In this work we provide a new estimation of the minimum growth temperatures of *S.* Enteritidis in raw and pasteurized whole egg, egg white and egg yolk through the use of probabilistic growth/no growth (G/NG) models and the inclusion of new variables/factors such as the initial concentration and the thermal history and storage temperature of the egg products.

Regarding the influence of the egg fraction, it is very well-known that egg white is a much more restrictive medium than whole egg and egg yolk (Guillén et al., 2021; Kim et al., 2018; Moon et al., 2016). The minimum growth temperatures for these egg products are in the range of those already published in the literature (ICMSF, 1996; Kang et al., 2021; Kim et al., 2018; Whiting and Buchanan, 1997), although it should be noted that, for both whole egg and egg white, the pasteurization enabled the growth of *Salmonella* cells at lower temperatures, according to our results. It should be also pointed out that differences (in the minimum growth temperatures) between whole egg and egg yolk were only found in some scenarios (see below). Thus, the minimum growth temperatures of *S.* Enteritidis cells would be similar in egg yolk (regardless of its thermal history), pasteurized whole egg and in raw whole egg when inoculated at high cell densities, being only higher in raw whole egg inoculated at low cell densities. The potential causes for these later two phenomena will be discussed below but the practical implications are obvious: while pasteurization treatments do ensure *Salmonella* inactivation, if these products are later contaminated, the probability (and rate) of *Salmonella* growth in pasteurized products would be higher than in raw ones.

Our results also demonstrate that in three of these products (raw whole egg and raw and pasteurized egg white) this temperature depends on the initial concentration. This is especially relevant because in most studies, a fixed inoculum level is used, without considering the potential effect of the initial concentration, though there is evidence suggesting that it may affect microbial growth (Koutsoumanis and Sofos, 2005; Masana and Baranyi, 2000; Pascual et al., 2001; Robinson et al., 2001). Thus, Koutsoumanis and Sofos indicated that cell density would have a significant impact on the growth/no growth interface and that the probability of growth would be significantly lower at lower cell densities (Koutsoumanis and Sofos, 2005). Similarly, Vermeulen et al. (2009) developed a growth/no growth model for L. *monocytogenes* that incorporated the influence of cell density, and validated a model developed for high cell concentrations with these data, finding to be invalid for lower cell densities. Results obtained in our work provide new evidence of the influence of the initial cell density on microbial growth (in this case on the minimum growth temperature of *Salmonella* Enteritidis) in egg products. This is of high relevance from an applied point of view since it indicates that models developed using high initial densities might not be applicable to low density scenarios, which are, on the other hand, the real (or most frequent) food scenarios and also because it reinforces the perception that the initial cell concentration should be considered when developing and using G/NG models to identify the conditions necessary to ensure an acceptable low-risk level. In any case, our results also indicate that this phenomenon (the influence of dose on microbial growth) would only happen in some food products, as will be discussed below.

Another relevant and differential aspect of our work/experimental design is that the experiments were carried out with monocultures (in this case 4 Enteritidis strains) while most of the models are constructed on the basis of results obtained with strain cocktails (Koutsoumanis et al., 2004; Valero et al., 2009; Vermeulen et al., 2007). Besides other potential advantages, this experimental design was chosen because it provides very valuable data regarding the intra-specific (in this case intra-serovar) variability at the low temperature growth boundaries. Thus, as mentioned throughout the manuscript, our results reveal the existence of substantial differences in the minimum growth temperatures depending on the strain studied, with differences of up to 5–6 °C in egg white In addition to the models developed for each strain, we also built for each egg product a model fitting the data of the 4 strains altogether (global models) and we compared the results obtained with those of the strains when modeled individually and also to the average minimum growth temperatures (for 3 fixed growth probabilities) of the 4 strains (average approach). As expected, the minimum growth temperature value calculated for a probability of 0.1 for the global model was similar to that of the strain displaying the lowest minimum growth temperature (STCC 4300 or 4155 depending on the egg product). Similarly, the minimum growth temperatures for a probability of growth of 0.9 calculated with this global model were similar to those determined for this same probability (0.9) for strain STCC 4396, the one needing higher temperatures for growth. In fact, it is reasonable to think that this model would probably be very similar to the one we would had obtained if we had inoculated a cocktail of these for carrying out the G/NG experiments. Accordingly, Gysemans et al. (2007) already observed, when comparing two types of logistic regression models from a case study with monoculture and mixed strain culture data, that the models providing a better fit were different depending on the experimental design (monoculture *vs* mixed culture).

By contrast, and as already indicated above, this global model generated a large transition zone than the one resulting after calculating the average minimum growth temperatures (of the 4 strains) for each growth probability. This is due to the higher influence that strains with extreme behaviors exert on the first one, which is also a good reflection of its benefits and drawbacks compared to the second. Thus, if maximizing food safety is the target this global model would be the best choice among these two, because it would estimate lower minimum temperatures for the lowest growth probabilities (although the opposite would also be right for the highest probabilities). By contrast, it should also be noted that the *Salmonella* Enteritidis minimum growth temperatures determined using this global model are highly influenced by strain selection (type and number). Thus, as already pointed out, if one of the strains selected for the study has a very extreme behavior, transition zones (a reflection of variability *S.* Enteritidis among serovars). This fact would be particularly relevant the lower the amount of strains included in the study and, therefore, our estimations for the whole serovar should be taken with care since they are based on only 4 strains.

It is also important to highlight that the models developed in this work were based on data obtained in the target food matrixes. Most of the existing G/NG models for *Salmonella* have been obtained in laboratory media (Basti and Razavilar, 2004; Koutsoumanis et al., 2004; Lanciotti et al., 2001), although subsequently validated in food. However, the complex composition of egg, specially egg white, makes difficult to develop laboratory media/models mimicking the egg product. For this reason, we opted to work in real foods, even though this implied that we had to determine if growth had occurred or not on the basis of single (or two in the case of egg white) measurements instead of using whole growth kinetics. It should also be noted that, in addition to food structure and composition, competitive microbiota in food has been proved to significantly affect *Salmonella* growth ability/rate (Oscar, 2008). However, it was found out that the internal contamination of the eggs used in this study was extremely low (data not shown) and much lower that the lowest dose inoculated in all cases. Therefore, this unlikely affects the estimations done, and would not help to explain the differences between strains, batches and/or products.

The main factor limiting bacterial growth in egg has long been considered to be iron restriction (Garibaldi, 1970; Schade and Caroline, 1944). Thus, although the concentration of iron in egg has been estimated to be between 3.6 and 18 μM, it is assumed that there would be no

free iron in egg white, since it would be chelated by ovotransferrin. To overcome this limitation in iron bioavailability (Dostal et al., 2014), *Salmonella* has developed different systems for the acquisition of iron including, among others, the ferric iron uptake system *via* siderophores. The main siderophores of *Salmonella* are salmochelins and enterobactins, which are characterized by their high affinity for iron. According to previous studies it seems that the synthesis of these siderophores is key for the growth of *Salmonella* in egg (Correnti et al., 2011; Julien et al., 2020) and furthermore, our previous studies suggest that the bioavailability of iron would explain the differences in growth rate between fractions, depending on the initial concentration and the thermal history of the egg (Guillén et al., 2021).

In that work it was demonstrated that the growth rate of *S.* Enteritidis cells depends on the thermal history of egg white and liquid whole egg but not egg yolk, and also, that it depends on the initial cell concentration for egg white (both raw and pasteurized) and raw liquid whole egg (Guillén et al., 2021). Results obtained in this work highly resemble those obtained previously since the minimum growth temperature of the 4 *Salmonella* strains here studied was only dose-dependent in egg white (both raw and pasteurized) and raw liquid whole egg, no differences between raw and pasteurized egg yolk were observed and the minimum growth temperature in raw liquid whole egg inoculated at high initial concentrations was similar to that in pasteurized liquid whole egg. Therefore, and considering all the above indicated, it is reasonable to speculate that iron bioavailability might also be determining the minimum growth temperatures of *Salmonella* in eggs and egg products.

On the other hand, and regarding the initial concentration dependence of the minimum growth temperatures observed in some cases (raw whole egg and egg white) it also seems plausible that iron bioavailabity might be playing a role since this phenomenon was only observed in those cases in which iron is restricted in the growth medium. Thus, this phenomenon might be explained on the basis of the hypothesis proposed by Scholz and Greenberg (2015), who proposed that, siderophores would be used as a private good at low cell density but that will be shared at high cell density. However, the higher ability of *Salmonella* cells to growth at low temperatures when inoculated at higher concentrations that was observed in some scenarios, might also be due to its larger likelihood of having fast growing cells, as proposed for explaining the influence of the initial concentration on lag phase duration by Akkermans and Van Impe (2021). It should be noted that these hypotheses are not necessarily exclusive and that, furthermore, other potential explanations cannot be ruled out. Further work will be required to clarify this point and elucidate the mechanisms responsible for the differences in minimum growth temperatures among *Salmonella* Enteritidis strains.

Finally, it should be noted that although our experimental approach has many benefits, it also has some limitations. Thus, and as in most studies of this type, cultures were obtained under fixed/standard conditions -in this case an iron rich medium (TSB-YE) and at optimum growth temperatures (37 °C)-, which can be far from the conditions encountered in practise. We did check that obtaining *Salmonella* cultures in raw liquid whole egg led to similar results to those here reported (data not shown) but it would also be very interesting to determine if pre-adapting *Salmonella* cells to low temperatures has any effect on their minimum growth temperatures in egg products and, if that is the case, to carry out new experiments in order to get a more accurate estimation of *Salmonella* growth boundaries in them.

5. Conclusions

The results presented in this article demonstrate that the minimum growth temperatures of *Salmonella* Enteritidis are higher in egg white than in egg yolk and whole liquid egg. Results also reveal that in some products, such as raw whole liquid egg and raw and pasteurized egg white, the minimum growth temperature of *Salmonella* Enteritidis cells is dependent on the initial dose. Similarly, the previous thermal history of the egg product affected the minimum growth temperature in some cases, notably whole liquid egg. However, no influence of dose or thermal history was observed in egg yolk. In egg white, the minimum growth temperatures were dose dependent and the influence of the thermal history would be small, if existing. Finally, pasteurization of whole liquid egg abolished the dose dependency of the minimum growth temperature, and therefore, differences in this parameter between raw and pasteurized whole liquid egg were only found when low inoculation doses were compared.

Our experimental design (with monocultures) allowed us to quantify the intra-specific variability in minimum growth temperatures among *S.* Enteritidis strains. In this sense, significant differences between strains were observed in certain products (up to approx. 6 °C in egg white), with one of them, STCC 4396, showing a markedly higher minimum growth temperature than the other three strains in almost all the egg products studied.

In summary, the models developed are capable of providing a more accurate prediction of *Salmonella* minimum growth temperatures in egg products by considering dose and thermal history, while also providing a quantification of the intra-specific variability. This is highly relevance for enhancing the safety of egg products since it would allow to develop more precise *Salmonella* quantitative risk assessments and also to improve their production, storage and distribution processes. Results here obtained might also help to revise or refine current guidelines for food safety management for both industry and consumers. Thus, as a way of example, our results indicate that none of the strains studied was capable of growing at 5 °C under any of the conditions assayed. Therefore, storage of egg products (particularly whole liquid egg and egg yolk) below this temperature might be regarded/proposed as a good management approach.

CRediT authorship contribution statement

Silvia Guillén: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Lara Domínguez:** Investigation, Writing – review & editing. **Pilar Mañas:** Formal analysis, Methodology, Supervision, Writing – review & editing. **Ignacio Álvarez:** Formal analysis, Methodology, Supervision, Writing – review & editing. **Elena Carrasco:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Guillermo Cebrián:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110619.

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