Stress resistance of emerging poultry-associated *Salmonella* serovars

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ABSTRACT

In recent years, the on-farm prevalence of some poultry-related *Salmonella* serovars such as *S.* Kentucky, *S.* Heidelberg, *S.* Livingstone and *S.* Mbandaka has increased significantly, even replacing *S.* Enteritidis and *S.* Typhimurium as the most frequently isolated serovars in some production settings and countries. For this reason, the aim of this work was to determine the resistance to several stressing agents and food preservation technologies, in laboratory media and in egg products, of 4 strains of these emerging *Salmonella* serovars associated to poultry and poultry products and to make comparisons with 4 *S.* Enteritidis strains. First, the resistance to acid pH, hydrogen peroxide, NaCl, heat, HHP, PEF and UV of the 8 *Salmonella* strains studied was determined and compared in laboratory media. From this part of the study, it was concluded that variability in resistance to stress among the 8 studied strains varied depending on the investigated agent/technology. However, differences in resistance (*2D*-values) were always lower than 3.3-fold. Results obtained also indicated that the strains of the emerging serovars studied would display lower acid and NaCl resistance, higher heat resistance and similar oxidative, HHP, PEF and UV resistance than *S.* Enteritidis. Then, the resistance of these 8 strains was evaluated and compared in egg, egg products and poultry manure. For some agents -including osmotic stresses, UV and PEF- there was a very good correspondence between the results obtained in laboratory media and in real food matrices and poultry manure (r>0.85; p<0.01). A significant relationship was also found for acid and HHP resistance (p<0.05) and a trend for heat resistance (p<0.10). Therefore, in general terms, conclusions drawn from the study carried out in laboratory media -regarding intraspecific variability and the relative resistance of the different strains- might be extrapolated, although with caution, to real food scenarios. Results obtained in this investigation would help to better understand the physiology and ecology of *Salmonella* and to design better egg preservation strategies.

Keywords: foodborne pathogens, egg, non-thermal technologies, variability.

1. INTRODUCTION

Salmonella is the most commonly reported causative agent of foodborne outbreaks in the European Union (EFSA, 2019) and constitutes one of the greatest public health concerns worldwide. The sources of *Salmonella* contamination are relatively diverse, but one of the most important sources is poultry and poultry products. Thus, eggs and egg products stand out as the most frequently identified source of foodborne *Salmonella* infections (45.6% of *Salmonella* outbreaks in Europe in 2018), followed by various types of meat and meat products (16.8%) (EFSA, 2019).

The serovars most frequently implicated in non-typhoid salmonellosis in humans are *S.* Typhimurium and *S.* Enteritidis. Approximately 71% of confirmed human cases in Europe in 2018 are attributed to *S.* Typhimurium and *S.* Enteritidis, almost 50 % of them corresponding to Enteritidis (EFSA, 2019). Similarly, they are also the most prevalent serovars, among the five included in the European National Control Programmes, 2007-2017, in *Gallus gallus* breeding flocks, with a prevalence of 0.25 % and 0.12 % positive flocks for *S.* Enteritidis and *S.* Typhimurium, respectively. Regarding eggs and egg products, it should be noted that most of the strong-evidence *Salmonella* food-borne outbreaks in the European Union involving them were linked to *S.* Enteritidis (66.7 % of cases versus 6.5 % of *S.* Typhimurium) (EFSA Panel on Biological Hazards (BIOHAZ), 2014). However, in spite of the predominant role that these two serovars play nowadays, it should be noted that this has not always been the case (Foley et al., 2011; Hennessy et al., 2004; Martelli & Davies, 2012). In fact, it is believed that the niche created by the eradication through sanitation efforts of the widespread serovars *Salmonella enterica* Pullorum and Gallinarum in the 1960s, conducted to the emergence of *S.* Enteritidis as the main serovar associated to eggs and egg products (Foley et al., 2011).

In recent years, the prevalence of serovars such as *S.* Kentucky and *S.* Heidelberg has increased significantly (EFSA, 2019; Kaldhone et al., 2017). Thus, in the United States of America *S.* Heidelberg replaced *S.* Enteritidis as the most frequently isolated poultry serovar from 1996 to 2006 and since 2007 it has been replaced by *S.* Kentucky (Foley et al., 2011). Similarly, in Europe, *S.* Mbandaka and *S.* Livingstone already exceed *S.* Enteritidis in frequency of isolation in broilers and *S.* Kentucky is the third most commonly found in laying hens, after *S.* Enteritidis and *S.* Infantis (EFSA, 2019). All these data indicate that, in many countries and poultry settings, these emergent serovars have already supplanted *S.* Typhimurium and *S.* Enteritidis as the most relevant serovars associated with poultry production, at least form a food production and animal health perspective. The potential causes underlying these population shifts have been discussed in detail by Foley and coworkers (2011).

Nevertheless, the data accumulated to date demonstrate that higher on-farm prevalence does not always imply a higher incidence of disease in humans, as these *Salmonella* serovars (e.g. Mbandaka and Livingstone) are associated with a low incidence in humans (Foley et al., 2008). Causes of this phenomenon have been partially explored although not completely identified. These studies have focused on the host specificity of the different strains and the mechanisms of egg contamination. These two aspects would explain, for example, the causes of the high incidence in humans of *S.* Enteritidis, which is not host-specific and, moreover, can be transmitted to the egg by transovarian route (Martelli and Davies, 2012). However, none of these reasons could explain why some serovars that are frequently isolated in chickens, such as *S.* Mbandaka or *S.* Livingstone (EFSA, 2019) have such a low incidence in humans, despite not being poultry specific serovars. Unluckily, in spite of their increasing relevance, information on these serovars is still scarce, especially regarding their stress resistance.

Therefore, the aim of this work was to determine the resistance to several stressing agents and food preservation technologies, in laboratory media and in egg products, of 4 strains belonging to emerging *Salmonella* serovars associated to poultry and poultry products and to compare it with that of *S.* Enteritidis strains.

2. MATERIAL AND METHODS

2.1 Bacterial strains

To carry out this investigation, 8 strains belonging to *Salmonella enterica* subsp. *enterica* were selected, 4 of them corresponding to *S.* Enteritidis. The strains of *S.* Enteritidis (STCC 4155, STCC 4396, STCC 7160 and STCC 7236) were supplied by the Spanish Type Culture Collection. *S.* Heidelberg DMS 9379 was supplied by the German Collection of Microorganisms. *S.* Kentucky NCTC 5799, *S.* Mbandaka NCTC 7892 and *S.* Livingstone NCTC 9125 were supplied by Public Health England. All strains were maintained frozen at -80 °C in cryovials for long-term preservation.

2.2 Growth conditions

Cultures were grown in 96 wells microtiter plates (Thermo Scientific, Roskilde, Denmark). They were prepared by inoculating 100 µL of tryptic soy broth (Oxoid, Basingstoke, UK) supplemented with 0.6 % w/v yeast extract (Oxoid; TSB-YE) with a single colony previously isolated on a plate of tryptone soy agar supplemented with 0.6% w/v yeast extract (Oxoid; TSA-YE). Microtiter plates were sealed with a polyester impermeable film (VWR International, Leuven, Belgium) and incubated overnight at 37 °C under static conditions. One µL of these pre-cultures was inoculated into 100 µL of fresh TSB-YE and incubated for 24 h under the same conditions to obtain the stationary growth phase cultures that were used for stress resistance determinations. Preliminary studies showed that growth fitness and stress resistance of *Salmonella* cells grown

following this methodology was comparable to that of cell suspensions obtained in conventional 250 mL flask under agitation (150 r.p.m). For some experiments, strains were also grown in commercial pasteurized liquid whole egg (Pascual, Aranda de Duero, Spain).

2.3 Inoculation of poultry products and poultry manure.

Medium-sized eggs (53-63 grams) were purchased from a local supermarket. The eggshells were thoroughly washed with 70 % ethanol, allowed to air dry, and held at room temperature for at least 1 h before each experiment. Eggshell inoculation procedure was similar to that described by Keklik, Demirci, Patterson & Puri, (2010). Ten µL of inoculum solution was spreaded on the top surface in an area of 2×1 cm rendering 7 to 8 Log_{10} CFU/cm^2 on the inoculated egg surface, approximately. To enhance the fixation of the cells, samples were kept under laminar flow in a biological hood for 30 min before the treatments.

Commercial pasteurized liquid whole egg (Pascual, Aranda de Duero, Spain) was inoculated at an initial concentration of 10^7 CFU/mL. The pasteurized liquid whole egg was characterized by measuring its pH, water activity and electrical conductivity. The pH was measured using a pHmeter BASIC 20 (Crison Instrument, Barcelona, Spain), water activity was measured at room temperature with a dew point instrument (Water Activity System mod. CX-1, Decagon Devices, Pullman, WA, USA) and electrical conductivity was measured with a FYA641LFP1 conductivity probe (Ahlaborn, Holzkirchen, Germany) connected to an Almemo 2590 data logger (Ahlaborn, Holzkirchen, Germany).

Commercial mayonnaise (1 g; Hellmann's Mayonesa Ecológica, Univeler España, Viladecans, Spain) was inoculated at an initial concentration of 10^7 CFU/g. This mayonnaise is mainly composed of oil (78%) and egg yolk (7.4%), is acidified to with

vinegar and lemon juice and has no other preservative added. Its pH and a_w were measured as described above.

The poultry manure was collected from a *Salmonella* free breeding flock, at the Faculty of Veterinary of the University of Zaragoza (Spain). Its pH and a_w were also measured as described previously. For the inoculation of poultry manure, 5 g of it were inoculated with 20 µL of the inoculum and mixed homogeneously (manually shaking within a Petri dish for 1 min), giving an initial concentration of 10^7 CFU/g.

2.4 Acid, hydrogen peroxide, and sodium chloride and desiccation resistance determinations

Resistance to chemical agents in laboratory media was carried out as described in Guillén et al. (2020). The treatment medium for acid-resistance determinations was citrate-phosphate McIlvaine buffer adjusted to pH 2.5 (Dawson et al., 1974). Hydrogen peroxide resistance was evaluated in 100 mM Tris–HCl buffer (pH 7.0) with hydrogen peroxide added at final concentration of 30 mM (Sigma, St Louis, USA). Resistance to osmotic medium was evaluated in TBS-YE supplemented with 30 % w/v of sodium chloride (a_w = 0.786 ± 0.01) (VWR International; NaCl). In all cases, treatments were performed on microtiter plates, and cells were added to the treatment medium to an initial concentration of 10^7 CFU/mL. After inoculation, the suspensions were incubated at a constant temperature of 25 ºC throughout the treatment, except for the NaCl determinations, which were carried out at 37 ºC due to the low lethality of this agent at room temperature (25 ºC). After the selected contact time, up to 50 min, 100 min and 32 h for acid, hydrogen peroxide and sodium chloride determinations, respectively, 20 µL samples were withdrawn at preset intervals and transferred into 180 µL of buffered peptone water (Oxoid; BPW). Subsequent serial dilutions were prepared and pour-plated for survival counts as described below.

Decontamination (washing) experiments in eggshells were carried out following the protocol of Cox, Mauldin, Kumararaj & Musgrove (2002), slightly adapted for our purposes. Briefly, treatments consisted in the application of 1.5 mL per egg of a solution of 2 % citric acid or 0.15 % hydrogen peroxide to the inoculated eggs with a fine mist sprayer. In parallel, the decontamination efficacy of washing with 1.5 mL of sterile distilled water was also tested, as a control. Treated eggs were air-dried for 1 h before microbiological sampling. For microbial recovery, eggs were gently broken, discarding the contents, and each eggshell was deposited in a sterile stomacher bag (VWR) containing 50 mL of BPW and homogenized for 30 s at 230 r.p.m in a stomacher laboratory blender (model 400, Seward, West Sussex, UK).

Salmonella resistance to acid conditions was also determined in acidified mayonnaise. After its inoculation, the mayonnaise samples were preserved for up to 12 h at room temperature. Microbial recovery was carried out as described for chemical agents using the laboratory media indicated above.

Resistance to desiccation was determined in two conditions by testing the viability of *Salmonella* strains after its inoculation in eggshells and in poultry manure. The inoculated eggshells and poultry manure were maintained at room temperature and under normal room atmosphere (RH=69-75%) for up to 24 and 72 h, respectively. Microbial recovery was carried out as described above. In the case of the poultry manure, 5 g were diluted in 45 mL of BPW.

2.5 Heat treatments

Heat treatments were carried out in a Mastia thermoresistometer (Conesa et al., 2009). Briefly, this instrument consists in a 400 mL vessel provided with an electrical heater for thermostation, a cooling system, an agitation device to ensure inoculum distribution and

194 temperature homogeneity, and ports for the injection of microbial suspension and for the
195 extraction of samples. The thermoresistometer was programmed to perform a linear
196 temperature profile from 25 to 58 or 60 ± 0.1 °C at a rate of 2 °C/min. Once treatment
197 temperature had attained stability, 100 µL of the microbial cell suspension was injected
198 into the main chamber containing the treatment media, tryptic soy broth or pasteurized
199 liquid whole egg. After inoculation, samples were collected at different heating times, up
200 to 5 min, and immediately pour plated and incubated for survival counting.

201 **2.6 High hydrostatic pressure (HHP) treatments**

202 HHP treatments were carried out in a Stansted Fluid Power S-FL-085-09-W (Harlow,
203 London, England) apparatus (Ramos et al., 2015). The pressure-transmitting fluid was a
204 mixture of propylene glycol and distilled water (50/50, v/v). An automatic device was
205 employed to set and/or record pressure and time during the pressurization cycle. Cell
206 suspensions were diluted to a cell concentration of 10^7 CFU/mL in citrate-phosphate
207 McIlvaine buffer of pH 7.0 or commercial pasteurized liquid whole egg. Samples were
208 packed in plastic bags, which were sealed without headspace and introduced in the
209 treatment chamber. Treatments were applied at 300 MPa for different treatment times up
210 to 30 min, and temperature never exceeded 40 °C.

211 **2.7 Pulsed electric field (PEF) treatments**

212 The PEF equipment used in this investigation was supplied by ScandiNova (Modulator
213 PG, ScandiNova, Uppsala, Sweden). The equipment and treatment chamber have been
214 previously described by Saldaña et al., (2009). Prior to PEF treatments, 100 µL of the
215 microbial cell suspension were dissolved in citrate-phosphate McIlvaine buffer (pH 7.0
216 and 1 mS/cm of electrical conductivity) or commercial pasteurized liquid whole egg (pH
217 7.5 ± 0.3 and a conductivity of 6.7 ± 0.3 mS/cm) at a concentration of approximately 10^7

CFU/mL. Samples were placed with a sterile syringe in the treatment chamber. Two different treatment chambers were used, one with a gap of 0.25 cm and an area of 2.0 cm^2 for treatments carried out in McIlvaine buffer and another with a gap of 0.4 cm and an area of 0.79 cm^2 for treatments in liquid whole egg. Treatments were based on square pulses with a width of 3 µs, applied at a frequency of 1 Hz for buffer treatments and at 0.5 Hz for egg treatments. Electric field strengths were set at 25 and 23 kV/cm. Under these experimental conditions, the energy per pulse was 1.88 and 5.63 kJ/kg for buffer and egg treatments, respectively. Treatments of up to 50 pulses (150 µs) were applied. Under these conditions, the final temperature of the treatment media was always below 35 °C.

2.8 Ultraviolet C light (UV-C) treatments

UV-C treatments were carried out in a microtiter plate under static conditions. Microtiter plates were coated with 1 layer of a microplate sealing film (BREATHseal, Greiner bio-one, Frickenhausen, Germany) and located at a distance of 22.50 cm from a 32 W UV-C lamp (VL-208G, Vilber, Germany). Radiation intensity was measured by means of a UVX radiometer (UVP, LLC, Upland, CA). Under these experimental conditions, an intensity of 0.47 ± 0.2 mW/cm^2 was attained. The treatment medium was citrate-phosphate McIlvaine buffer of pH 7.0, and the initial concentration was of 10^7 CFU/mL approximately. Treatment times of up to 120 s were applied and temperature never exceeded 30 °C. The surface-inoculated eggs were exposed to 6.36 ± 0.2 mW/cm^2 up to 15 s, giving a fluence of 0.10 J/cm^2. After its exposure to UV-C light, microbial recovery was carried out as indicated above.

2.9 Recovery after different treatments and survival counting

After treatments, all samples were adequately diluted in BPW and plated in the recovery medium, which was TSA-YE for all the samples but for those of poultry manure that were plated in in Xylose Lysine Desoxycholate Agar (Oxoid; XLD). Plates were incubated for 24 h (48 for XLD agar) at 37 °C, after which the number of colony forming units (CFU) per plate was counted.

2.10 Curve fitting and statistical analysis

All the determinations were carried out by triplicate in different working days. Survival curves (including at least 5 data points) were obtained by plotting the logarithm of the survival fraction (Log_{10} N/N_0) versus treatment time (hours for NaCl determinations; minutes for acid, heat, HHP, and peroxide treatments; seconds for UV treatments, and microseconds for PEF treatments). Since deviations from linearity were observed in survival curves to the majority of agents/technologies, the Geeraerd inactivation model-fitting tool from GInaFiT 1.7 (KU Leuven, Belgium), was used to fit survival curves and calculate resistance parameters. Eq. 1 is used to described survival curves with shoulder and Eq. 2 for those with tail.

$$N_t = N_0 \cdot exp^{-K_{max} \cdot t} \cdot \left[\frac{exp^{K_{max} \cdot S_l}}{1+\left(exp^{K_{max} \cdot S_l}-1\right) \cdot exp^{-K_{max} \cdot t}}\right] \quad \text{(Eq. 1)}$$

$$N_t = (N_0 - N_{res}) \cdot exp^{-K_{max} \cdot t} + N_{res} \quad \text{(Eq. 2)}$$

In these equations, N_t represents the number of survivors, N_0 the initial count, and t the treatment time.

This model describes the survival curves by means of three parameters: shoulder length (S_l), defined as the time before exponential inactivation begins; inactivation rate (K_{max}), defined as the slope of the exponential portion of the survival curve; and N_{res} which

describes residual population density (tail). Therefore, the traditional decimal reduction time value (*D*-value) can be calculated from the K_{max} parameter using equation 3.

$$D\text{-value} = 2.303/K_{max} \quad \text{(Eq. 3)}$$

Goodness of the fits of Eq. 1 and 2 were estimated through R^2 and RMSE calculated with Excel software. Standard deviations (SD) and Pearson's and Spearman correlation coefficient were calculated using GraphPad PRISM® statistical software (GraphPad Prism version 7.00 for Windows, GraphPad Software, San Diego, California, USA). The same software was used to carry out the Iterative Grubbs' test (Alpha = 0.05) and the statistical analyses (Welch's t-test, student t-test and ANOVA; *p*-value< 0.05).

3. RESULTS AND DISCUSSION

The resistance against seven different preservation technologies and environmental stresses of 4 strains belonging to emerging *Salmonella* serovars associated with poultry and poultry products has been evaluated in this study and subsequently compared with that of 4 strains of *S.* Enteritidis. The adequacy of the methodology used has already been discussed in Guillén et al., (2020). It was previously checked that the resistance of *Salmonella* cells to all the studied agents was similar regardless if they were grown in pasteurized liquid whole egg or in TSB-YE (data not shown). Therefore, for methodological reasons, mainly because liquid whole egg could not be sterilized, all the experiments were carried out with cells grown in TSB-YE. Survival curves to the 7 agents were obtained by plotting the logarithm of the survival fraction *vs* the treatment time, displaying different profiles. These profiles showed deviations from linearity, as an example, the survival curves for hydrogen peroxide and UV showed shoulders, while those for NaCl and PEF displayed tails. Therefore, in order to accurately describe them, the non-linear Geeraerd model (Geeraerd et al., 2000), was used to calculate the

corresponding resistance parameters (N_0; S_l; K_{max}, N_{res}). The mean values obtained for these parameters and their standard deviation, along with the goodness-of-fit parameters, are included in Table 1. The traditional decimal reduction time value (*D*) of each survival curve was calculated from its corresponding K_{max} (Eq. 3). It was decided to use the *2D*-value parameter (time required to inactivate the first 2-Log_{10} cycles) in order to establish meaningful comparisons among strains and/or agents as described in Guillén et al. (2020). Since the *2D*-values obtained for each agent/technology cannot be directly compared because of the different time scale of survival curves, for comparison purposes the calculated resistance parameters were normalized by dividing them by the average *2D*-value of the resistance of all the *Salmonella* Enteritidis strains here studied.

3. 1 Resistance to stressing agents and food preservation technologies in laboratory media

Table 1 includes the resistance parameters (S_l; K_{max}, N_{res} and *2D*- values) to the 7 different agents/technologies studied of the 8 strains (4 strains of *S.* Enteritidis, *S.* Heidelberg, *S.* Kentucky, *S.* Mbandaka and *S.* Livingstone) studied. As a way of example the *2D*-values to acid pH (2.5) for the 8 strains of *Salmonella* varied from 17.53 to 45.88 min, being *S.* Kentucky the most sensitive, and *S.* Enteritidis 7160 the most resistant one. In fact, other studies have shown that *S.* Kentucky was more sensitive to acid stress than the serovars Enteritidis and Mbandaka when exposed to the media at a pH of 2.5 (Joerger et al., 2009). Results obtained for all the agents studied are in the range of those previously obtained following the same methodology (Guillén et al., 2020). Only some particular behaviors are worth being noted, such as the low resistance to NaCl observed for *S.* Heidelberg, the barosensitivity of two strains of *S.* Enteritidis, 4155 and 4396, and of *S.* Mbandaka, the high thermotolerance of *S.* Kentucky and *S.* Livingstone and the high UV-C resistance of *S.* Mbandaka and *S.* Livingstone, as compared with previous studies (Guillén et al., 2020).

313 Variability in resistance among the 8 strains varied depending on the technology
314 investigated. As can be deduced from Table 1 and also from Figure 1 (see below) the
315 lower variability in resistance was found for H_2O_2 resistance and the highest for HHP
316 resistance. Thus, up to a 3.3-fold difference in resistance to HHP (*2D* values) between the
317 most and least resistant strains was observed. By contrast the *2D*-value of the most H_2O_2
318 resistant strain was only 1.2-fold higher than that of the most sensitive one. These ranges
319 are similar to those reported for *Salmonellae* in Guillén et al. (2020), and also to those
320 reported for heat resistance in the meta-analysis carried out by van Asselt & Zwietering
321 (2006) and in the study of Lianou and Koutsoumanis (2013), but lower than those reported
322 by the later authors for acid resistance (up to 6-fold).

323 To determine whether any positive or negative association could be found among
324 *Salmonella* resistance to the different stresses, Pearson's and Spearman's correlation tests
325 were conducted (Table 2). Before, the iterative Grubbs's test was carried out to detect
326 possible outliers. Grubbs's test identified a unique outlier: the *2D*-value to osmotic
327 medium of *S.* Heidelberg. Therefore, this value was excluded from further analysis.
328 For this set of strains, a positive correlation was found between resistance to PEF and
329 osmotic stress (Pearson r= 0.792, p-value= 0.034; Spearman r_s= 0.929, p-value= 0.007)
330 and between UV and heat resistance (Pearson r= 0.737, p-value= 0.037; Spearman r_s=
331 0.786, p-value= 0.028). In the previous study these positive correlations were not
332 observed (Guillén et al., 2020). On the other hand, correlations between UV and PEF
333 resistance and between NaCl and H_2O_2 resistance observed in our previous work (Guillén
334 et al., 2020) were not observed for the set of strains used in the present study. These
335 differences might be attributed to different factors, most probably the different number of
336 strains tested and the particular characteristics of the strains included in each set. Thus, in
337 order to obtain a wider and more robust view, the same statistical analysis was carried out

including all the strains (15 from the previous study + 8 included in the present study). Results obtained indicate that if the 23 strains are included in the comparison, a positive correlation between PEF and NaCl (Pearson r= 0.507, p-value= 0.016; Spearman r_s= 0.625, p-value= 0.002) and between H_2O_2 and NaCl (Pearson r= 0.629, p-value= 0.002; Spearman r_s= 0.559, p-value= 0.008) resistance would exist, but the relationship between PEF and UV and between UV and heat resistance turned to be non-significant. In any case, all these conclusions should be taken with care given the relative low number of strains studied. Potential explanations for the existence or absence of these correlations have been given elsewhere (Guillén et al., 2020).

Figure 1 illustrates the differences in resistance between the emerging *Salmonella* serovars and *S.* Enteritidis. The normalized resistance values to each agent/technology were calculated as described in materials and methods. In this figure, the resistance of *S.* Enteritidis is depicted in a box and whiskers format, whereas the resistance of the other serovars is included as data points. As can be observed in the figure, emerging serovars (as a cluster) tended to be less acid, NaCl and PEF resistant and more heat resistant than *S.* Enteritidis strains. However, clear deviations from this general trends were observed, such as the PEF resistance of *S.* Livingstone, which was comparable to that of *S.* Enteritidis strains. Further comparison (Welch test) of the resistance parameters calculated for each of the emerging serovars *vs* that of *S.* Enteritidis (the 4 strains considered together) revealed that the pH resistance of the 4 emerging serovars and the PEF resistance of 3 strains (Heidelberg, Kentucky and Mbandaka) was significantly lower (p <0.05) than that of *S.* Enteritidis, in line with the results indicated above. In addition, *S.* Kentucky and *S.* Livingstone strains were found to be significantly more heat resistant, *S.* Heidelberg significantly less NaCl-resistant and *S.* Mbandaka significantly less HHP-resistant and more UV-resistant than *S.* Enteritidis strains.

Altogether, results obtained in laboratory media indicate that, in general terms, the strains of the emerging serovars would display a lower acid and NaCl resistance, a higher heat resistance and similar oxidative HHP, PEF and UV resistance than *S.* Enteritidis cells. However, given the fact that only one strain of each serovar was studied, what it is not representative of the whole serovar and also results in comparisons –*vs S.* Enteritidis- with a different number of samples/replicates (3 vs 12), these conclusions should be taken with care. Further work will be required in order to validate these conclusions but, if these results are verified, they offer a potential explanation for the low incidence in humans of these emerging serovars. Thus, in spite of their high on-farm prevalence, their lower resistance to osmotic stress (commonly encountered in surfaces such as the eggshell) and, especially acid stress (which they will face in the stomach) would limit the number of cells reaching the gut and, therefore, the risk of illness. Nevertheless, it is also plausible these emerging serovars might be lacking some virulence gene/s that would play a role in human diseases but that are not necessary to colonize chickens or that they would have a lower ability to use some metabolites, thus making them unable to overcome the microbiota present in the intestine of mammals. In fact, Dhanani et al., 2015, observed that the four *S.* Kentucky strains they studied lacked several SPI2-associated genes, and suggested that this might explain in part their inability to induce diseases in humans. Furthermore, as observed by Shah (2014) it is also plausible that these stress sensitive strains would also display an impaired expression of virulence genes. Future studies examining the genetic and metabolic differences between serovars isolated in chickens and humans are needed in order to elucidate why certain serovars are associated with different hosts.

Given the relevance of these results, in the second part of this investigation the resistance to the different agents/technologies here studied of these strains belonging to emerging

388 *Salmonella* serovars was compared to that of *S.* Enteritidis in eggs and egg products.
389 Poultry manure was also included as it is a very relevant source of contamination and
390 infection of eggs and laying hens, respectively.

391 **3. 2 Survival in poultry manure**

392 Figure 2 includes the Log_{10} cycles of inactivation of the 8 strains after a fixed incubation
393 time (2 days) in poultry manure (pH=8.42 ± 0.06 and a_w=0.857 ± 0.02). Survival varied
394 widely depending on the strain (between 0.57 and 2.59 Log_{10} cycles of inactivation)
395 (Figure 2). *S.* Heidelberg was the most sensitive, and *S.* Enteritidis 4155, *S.* Enteritidis
396 7160, *S.* Enteritidis 7236 and *S.* Kentucky (no statistically significant differences among
397 these four; $p>0.05$) the most resistant strains. Thus, a variation of more than 4-fold
398 between the most and least resistant strains was observed (Figure 2). In general terms, it
399 can be concluded that Enteritidis strains survived better than emerging serovars in
400 manure.
401 In order to compare these data with those obtained in laboratory media, the following
402 calculations were done. *S.* Enteritidis STCC 4155 inactivation after 2 days in poultry
403 manure was taken as the reference value (1.18 Log_{10} cycles). With this value and the
404 inactivation parameters previously determined for this strain in NaCl-added laboratory
405 media (Table 1) the time required to achieve the same level of inactivation (1.18 Log_{10}
406 cycles) for this strain in NaCl-added media was calculated. Then, this time (5.27 h) was
407 used to calculate the Log_{10} cycles of inactivation attained for each of the other 7
408 *Salmonella* strains studied. These calculations enabled us to make direct comparisons
409 (Log_{10} cycles of inactivation in NaCl-added media *vs* Log_{10} cycles of inactivation in
410 poultry manure) between treatments of a similar lethality. The same procedure was
411 followed to establish the comparisons between laboratory and food products for all the
412 other agents/technologies here studied.

A strong correspondence between these data and those obtained in laboratory media was observed (Pearson r= 0.859, *p-value*= 0.006). This correlation could be explained by the fact that in both cases the water activity (0.786 for NaCl-added media and 0.857 for poultry manure) was well below the *Salmonella* growth boundaries. Regarding poultry manure, the water activity was not so low (0.857) but the pH, among other factors, might also be contributing to *Salmonella* inactivation. The results obtained by Himathongkham et al., (2000) were comparable with those obtained in this study. A lower survival capacity in manure could explain the lower incidence of emerging strains in humans, while the higher survival capacity of *S.* Enteritidis would be a hazard as a source of contamination of eggs and chickens.

3.3 Survival in eggshells and resistance to eggshell decontamination processes

The differences in resistance (Log_{10} cycles of inactivation) in eggshells and to eggshell decontamination processes such as acid and hydrogen peroxide washing and UV-light, of the 8 *Salmonella* strains studied is shown in Figure 3. As can be observed in Figure 3A, the number of Log_{10} cycles of *Salmonella* inactivated 24 h after their inoculation in the surface of eggshells varied from 0.71 to 2.88 depending on the strain/serovar studied. As in poultry manure *S.* Heidelberg was among the most sensitive strains being its resistance significantly lower than that of *S.* Enteritidis 7160, *S.* Enteritidis 7236 and *S.* Livingstone. The variability in resistance was also similar to that observed in manure (aprox. 4-fold), and a very good correlation between survival in eggshell and NaCl resistance (r= 0.867, p-value= 0.005) was found.

Eggshell decontamination, especially for hatching eggs, is critical to the poultry industry in terms of reducing the horizontal transmission of *Salmonella* in the laying house (Cox et al., 2000). Acid and hydrogen peroxide washing and UV-light have been widely demonstrated to be effective methods to eggshell decontamination. Furthermore, UV-

light has even been proposed as an alternative to chemical agents given its minimal negative impact on hatchability and cuticle (Al-Ajeeli et al., 2016; Cox et al., 2007; Melo et al., 2019).

Reductions in the number of viable *Salmonella* cells attached to the eggshell between 0.66 and 1.98 Log_{10} cycles for citric acid washings and between 0.55 and 2.13 Log_{10} cycles for peroxide washings were observed depending on the strain/serovar (Figures 3B and 3C). A similar result was obtained by Melo et al. (2019), who reported a reduction of 0.84 logarithmic cycles in the number of total microorganisms present in the eggshell after the application of a hydrogen peroxide solution, using a protocol similar to ours.

No correlation was found between the data obtained in laboratory media and washing experiments. In addition, whereas the variability is H_2O_2 resistance among *Salmonella* strains was very low, that to H_2O_2 washings was almost comparable to that of acid washings. These differences might be attributed to different factors/phenomena. For instance, it should be reminded that whereas freshly grown cells were used in the inactivation experiments carried out in laboratory media, in eggshell washing experiments the cells were previously inoculated and dried in the surface of the eggshell, imposing a desiccation stress to *Salmonella* cells that, as described above, would affect them to a different extent depending on the strain.

On the other hand, the number of Log_{10} cycles of inactivation attained after an UV treatment, 0.10 J/cm^2, varied between 1.38 and 3.16, for *S.* Mbandaka and *S.* Enteritidis 4396, respectively (Figure 3D). This results on eggs are in agreement with several previous reports, in which reductions from 0.60 to 3.24 were observed after the application of UV at a fluence of 0.10 J/cm^2 (Chavez et al., 2002; Holck et al., 2018). It should be noted that in spite of the facts that *Salmonella* cells were also exposed to a desiccation stress and that the intensity applied in eggshell experiments was more than

10-fold higher than in buffer experiments (6.36 *vs* 0.47 mW/cm^2), a good correspondence was found between the results obtained in buffer and in eggshell decontamination experiments (r= 0.953, *p-value*= 0.0002). Despite this strong correlation, the variability in resistance among the strains on the eggshell surface was nearly 2-fold higher than that observed in liquid medium (Table 1).

3.4 Survival in mayonnaise

Acidity is probably the most important intrinsic factor determining *Salmonella* survival in mayonnaise. In this case, the pH of the mayonnaise was pH 3.81 ± 0.3 (and its a_w=0.937 ± 0.01) and the acidulants included were acetic and citric acid. As can be observed in Figure 4 *Salmonella* counts after 12 h of incubation (25 ºC) in mayonnaise decreased from 1.33 to 2.20 Log_{10} cycles. Variability between strains/serovars was 1.65-fold, with *S.* Enteritidis 4396, *S.* Enteritidis 7169 and *S.* Enteritidis 7236 showing the highest tolerances and *S.* Livingstone the lowest. *S.* Enteritidis strains tended to display a higher resistance in mayonnaise than the emerging serovars, similarly to that observed in acid buffer (Table 1). A strong correlation was obtained between the results obtained in mayonnaise and those in acidic buffer (r= 0.724, *p*-value= 0.042). Similar results were found by Zhu and coworkers, who observed that a mixture of *S.* Enteritidis strains was significantly more resistant than a mixture of strains belonging to different serovars of *Salmonella*, including Heidelberg, in acidified mayonnaise with citric acid and acetic acid (Zhu et al., 2012).

3.5 Resistance to liquid egg decontamination/pasteurization technologies

A part of the egg production is intended for the manufacture of liquid egg. In the egg industry, the microbiological safety of liquid products is ensured mainly by heat pasteurization (Lechevalier et al., 2017; Silva and Gibbs, 2012) but other alternatives are being considered, such as PEF or HHP treatments (Monfort et al., 2010, 2012). Thus, in

488 the final part of this work the resistance of the 8 *Salmonella* strains to these 3 technologies
489 (heat, PEF and HHP) when treated in liquid whole egg was determined and compared.
490 The pH and a_w of this liquid whole egg were 7.5 ± 0.3 and 0.996 ± 0.01 respectively, and
491 its electrical conductivity was 6.7 ± 0.3 mS/cm.
492 First, it should be noted that the resistance of all the *Salmonella* strains studied to these
493 three technologies was higher in liquid egg that in buffer, indicating that the complexity
494 of the composition of liquid whole egg would exert a protective effect, as already
495 described elsewhere (Cebrián et al., 2016). Furthermore, our results indicate that this
496 protective effect would outbalance the sensitization effect that some antimicrobial egg
497 components, such as lysozyme, might induce on *Salmonella* cells (Liang et al., 2002;
498 Masschalck et al., 2001; Smith et al., 2002).
499 Figure 5A shows the number of Log_{10} cycles inactivated after 2 min at 60 ºC in liquid
500 whole egg. As can be observed, up to 2.71 Log_{10} cycles of inactivation were attained, for
501 *S.* Enteritidis 4155. Our results are similar to those obtained by Gurtler et al., (2015) in
502 liquid egg. Thus, in both studies *S.* Heidelberg was the most heat-sensitive strain,
503 followed by *S.* Mbandaka. Enteritidis strains showed a heterogeneous profile. When
504 comparing data obtained in buffer and in liquid whole egg, a Pearson correlation
505 coefficient of 0.701 (*p*-value= 0.053) and a Spearman correlation coefficient of 0.738 (*p*-
506 value= 0.046) were obtained. This indicates that, at least, a trend towards an association
507 between both parameters (heat resistance in buffer and in liquid whole egg) would exist.
508 In any case, these results also suggest that the protective effect exerted by liquid whole
509 egg might be different depending on the strain/serovar studied. In any case, given the low
510 number of strains studied and limited treatment conditions explored (a single temperature
511 in each medium) these conclusions should be taken with care and further work will be

512 required in order to fully elucidate if trends observed in buffer can be extrapolated to more
513 complex media such as liquid whole egg.
514 Regarding PEF, *Salmonella* inactivation in liquid whole egg after 60 μs at 23 kV/cm
515 ranged from 1.18 to 2.32 Log_{10} cycles, these values correspond to *S.* Heidelberg and *S.*
516 Livingstone respectively (Figure 5B). The variability in resistance among the studied
517 *Salmonella* strains was very low, less than 2-fold, similarly to that observed when they
518 were treated in laboratory media (2.1-fold). These results are similar to those obtained by
519 Monfort et al. (2010) who reported reductions around 2 Log_{10} cycles, after treatments at
520 20 and 25 kV/cm in liquid whole egg for *S.* Typhimurium, but higher than those observed
521 by Hermawan et al. (2004). As for osmotic and acid stresses and UV treatments, a
522 significant correlation was found between resistance to PEF in McIlvaine and in liquid
523 whole egg (r= 0.914, p-value= 0.002). Thus, in general, in both media, *S.* Enteritidis
524 strains showed a slightly higher resistance than the emerging strains.
525 The lethality of HHP treatments of 20 min at 300 MPa varied between 1.15 and 3.48
526 Log_{10} cycles of inactivation, for *S.* Enteritidis 4396 and *S.* Enteritidis 7236, respectively,
527 as depicted in Figure 5C. Thus, as described for the treatments in buffer, differences in
528 HHP resistance among the 8 strains in liquid whole egg varied approximately 3-fold.
529 Furthermore, a significant correlation was also found between the baro-resistance of
530 *Salmonella* in buffer and in liquid whole egg (r= 0.762, p-value= 0.028).

531 **4. CONCLUSIONS**

532 From the first part of the study, it can be concluded that variability in resistance among
533 the eight strains studied varied depending on the technology investigated. However,
534 differences in resistance (*2D*-values) were always lower than 3.3-fold. Our results
535 indicate that the strains of the emerging serovars studied would display a lower acid and

NaCl resistance, a higher heat resistance and similar oxidative, HHP, PEF and UV resistances than *S.* Enteritidis.

For some agents, including osmotic stresses, UV and PEF, there was a very good correspondence between the results obtained in laboratory media and in real food matrices and/or poultry manure (r>0.85; p<0.01). A significant relationship was also found for acid and HHP resistance (p<0.05) and a trend for heat (p<0.10). Therefore, in general terms, conclusions drawn from the study carried out in laboratory media -regarding intraspecific variability and the relative resistance of the different strains- might be extrapolated, although with caution, to real food scenarios. Further work would be required in order to fully elucidate if this is also true for heat treatments.

Results obtained in this investigation would help to better understand the physiology and ecology of *Salmonella* and to design better egg preservation strategies. It is noteworthy that the lower acid and osmotic stress resistance of these emergent serovars might explain their relatively low incidence in humans. However, this hypothesis is based in the results obtained only with a single strain of each serovar and further work will be required to validate it.

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AUTHORS CONTRIBUTIONS

559 **Silvia Guillén:** Investigation, Methodology, Formal Analysis, Writing-Original draft
560 preparation. **María Marcén**: Investigation, Writing - Review & Editing. **Ignacio**
561 **Álvarez**: Methodology, Writing - Review & Editing. **Pilar Mañas**: Conceptualization,
562 Writing - Review & Editing. **Guillermo Cebrián**: Conceptualization, Writing - Review
563 & Editing, Supervision.

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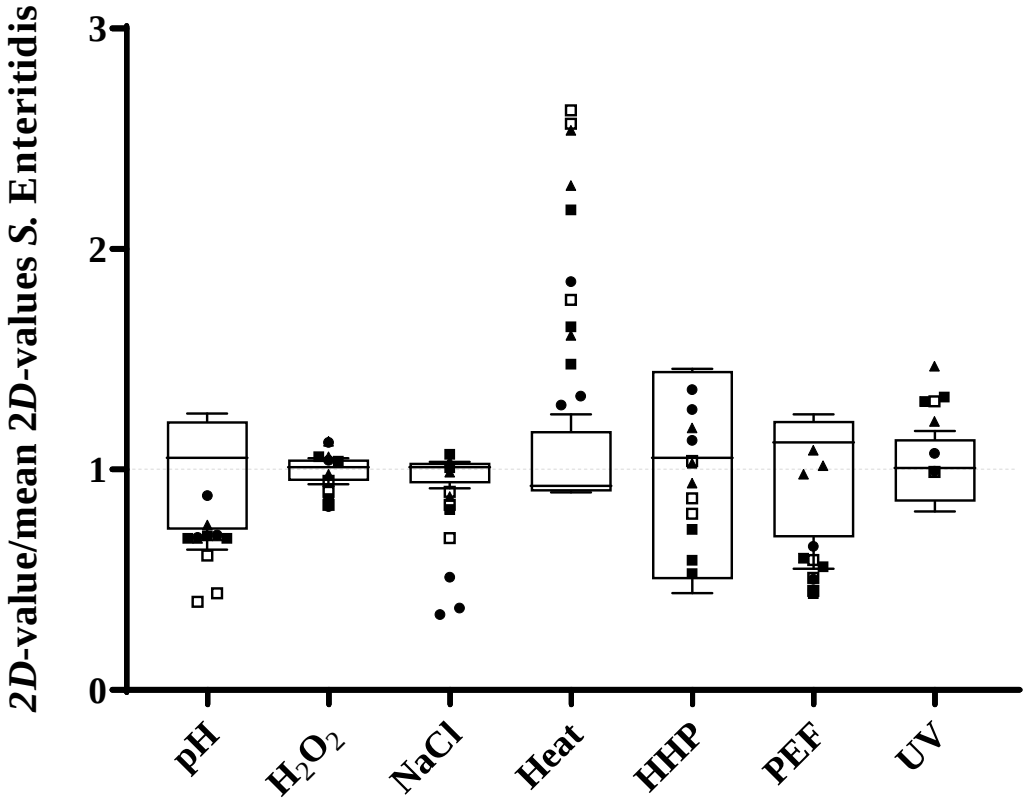


Figure 1. Variability in resistance (expressed as the ratio between the *2D*-value calculated for each strain and biological replicate and the mean *2D*-value of all *S.* Enteritidis strains) to different environmental stresses and non-thermal food preservation technologies among the *Salmonella* strains studied. The boxes depict the variability among the 4 *S.* Enteritidis strains tested and the points (each one corresponding to a biological replicate) correspond to the values calculated for each emerging poultry-associated *Salmonella* serovar: *S.* Heidelberg ●, *S.* Kentucky □, *S.* Mbandaka ■ and *S.* Livingstone ▲.

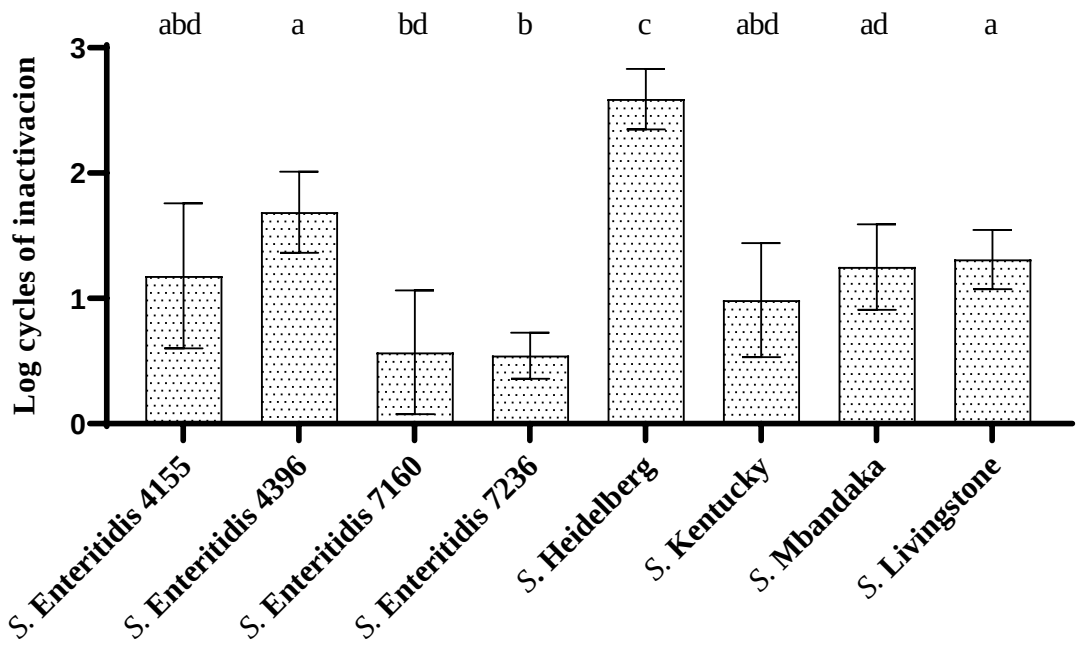
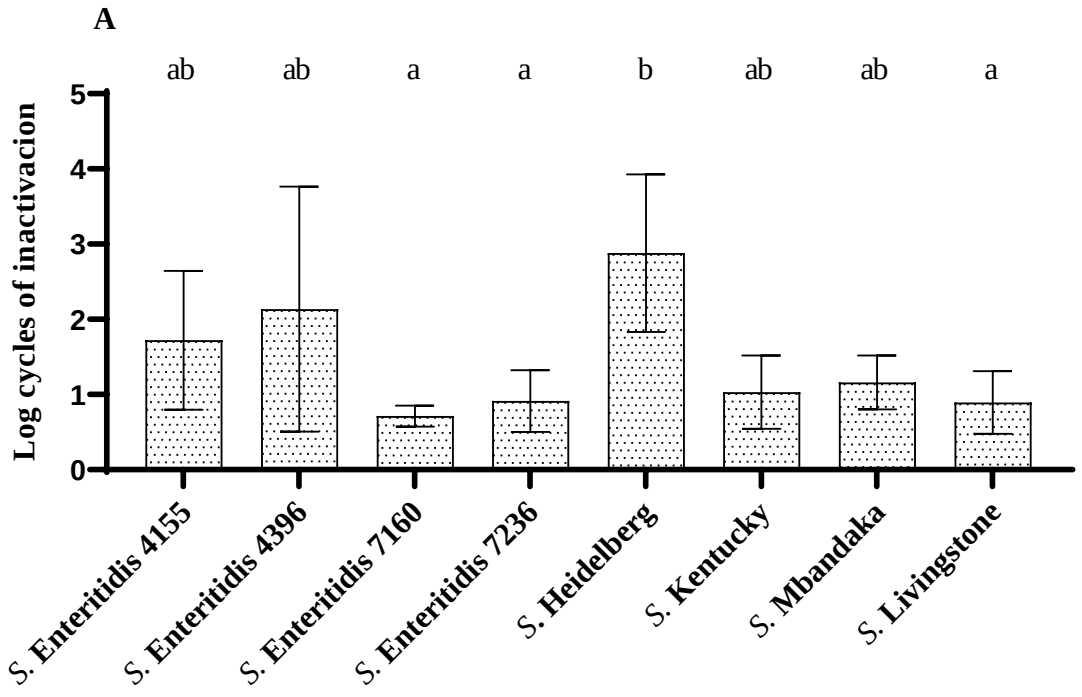


Figure 2. Log cycles of inactivation after 2 days of incubation in poultry manure (25 ºC; pH 8.42; a_w=0.857) of 8 the *Salmonella* strains studied. Different letters indicate statistically significant differences between strains. Error bars correspond to the standard deviation of the means.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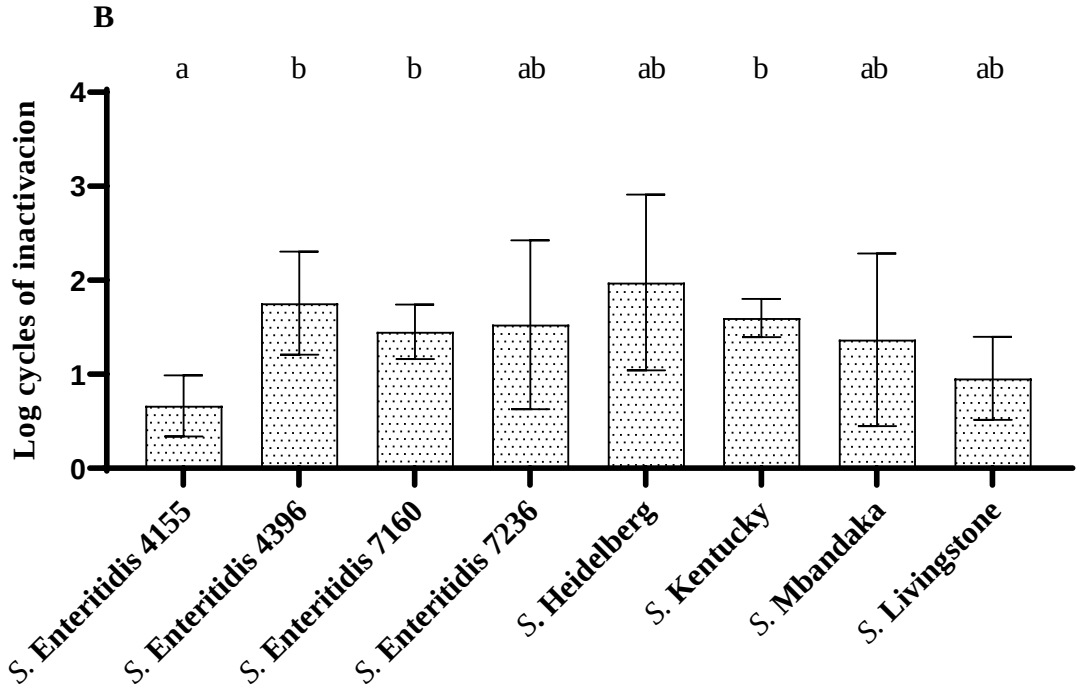
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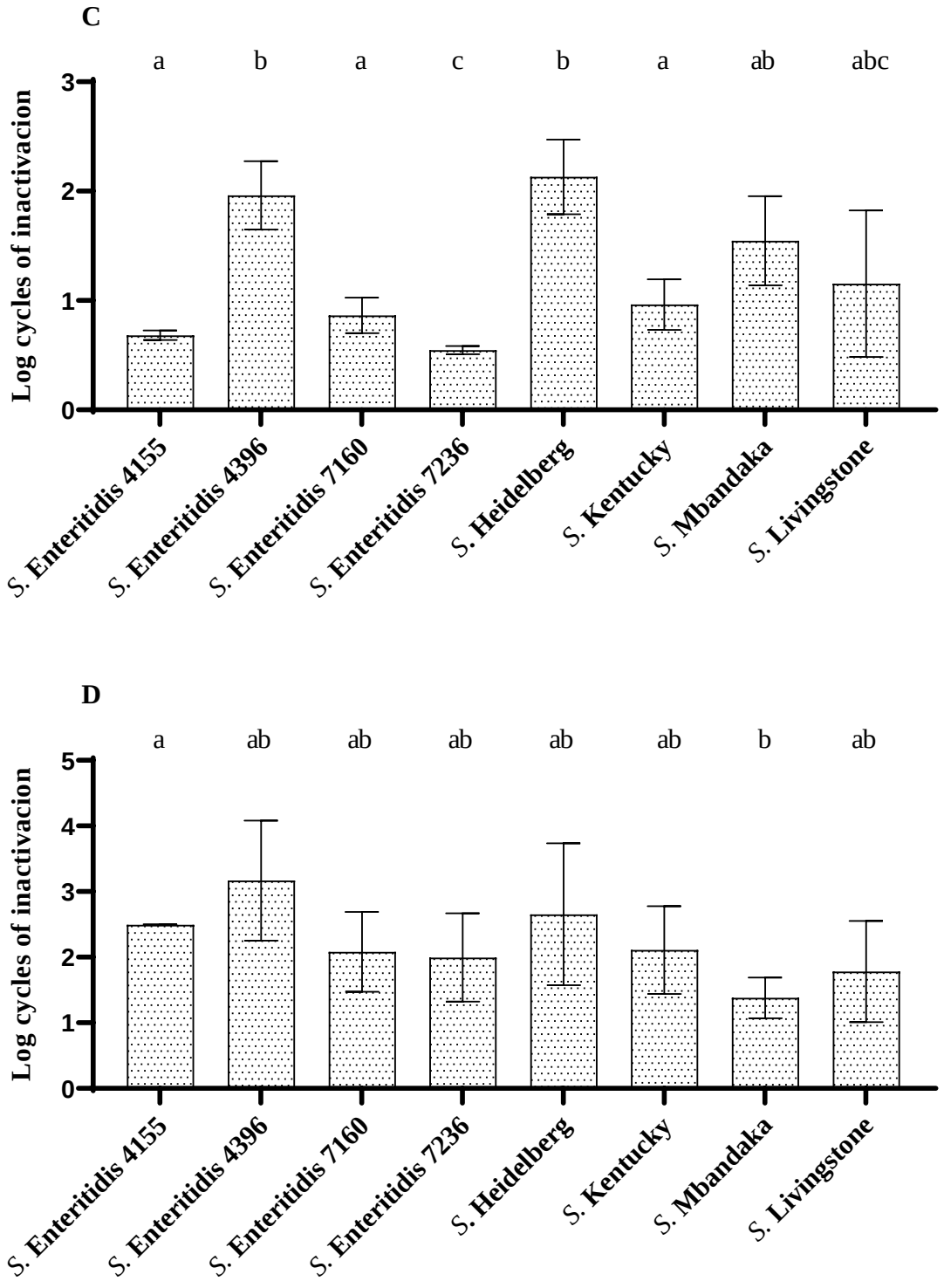


Figure 3. Survival in eggshells and resistance to eggshell decontamination processes of the 8 *Salmonella* strains studied. A) Log cycles of inactivation in eggshell after 24 hours (25 °C, RH=69-75%). B) Log cycles of inactivation after citric acid (2 %) washing. C) Log cycles of inactivation after hydrogen peroxide (0.15 %) washings. D) Log cycles of

inactivation after UV treatments (6.36 mW/cm^2). Different letters indicate statistically significant differences between strains. Error bars correspond to the standard deviation of the means.

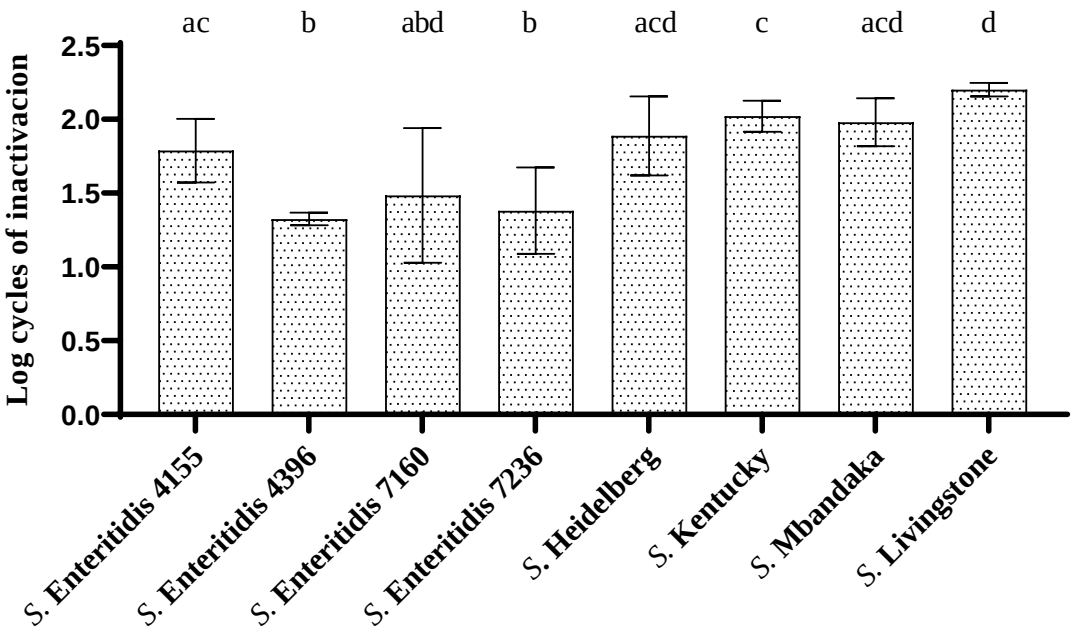
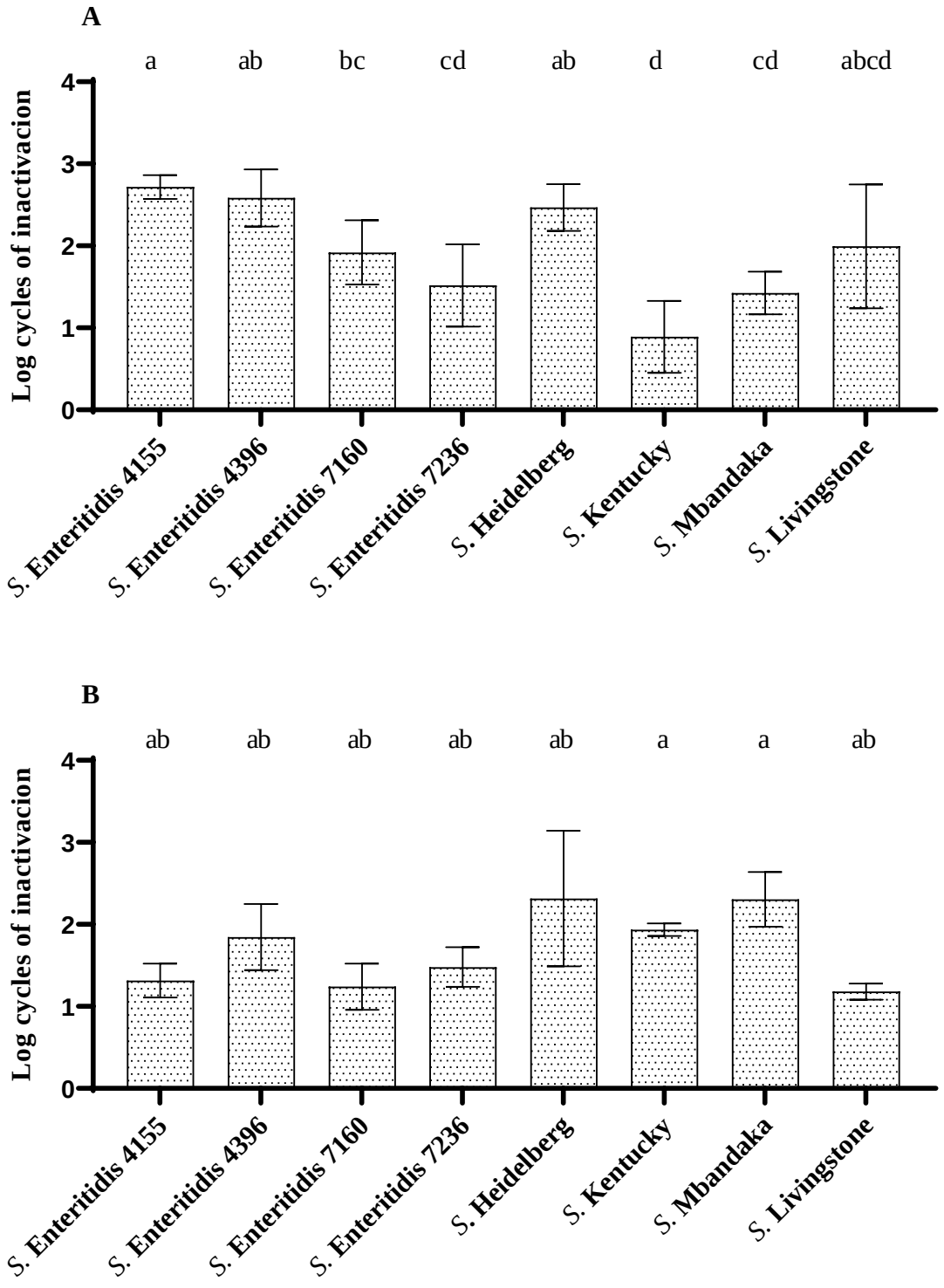


Figure 4. Log cycles of inactivation after 12 hours of incubation in acidified mayonnaise (25 ºC; pH 3.81) of 8 the *Salmonella* strains studied. Different letters indicate statistically significant differences between strains. Error bars correspond to the standard deviation of the means.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B
ab
ab
ab
ab
ab
a
a
ab
Log cycles of inactivacion
0
1
2
3
4
S. Enteritidis 4155
S. Enteritidis 4396
S. Enteritidis 7160
S. Enteritidis 7236
S. Heidelberg
S. Kentucky
S. Mbandaka
S. Livingstone

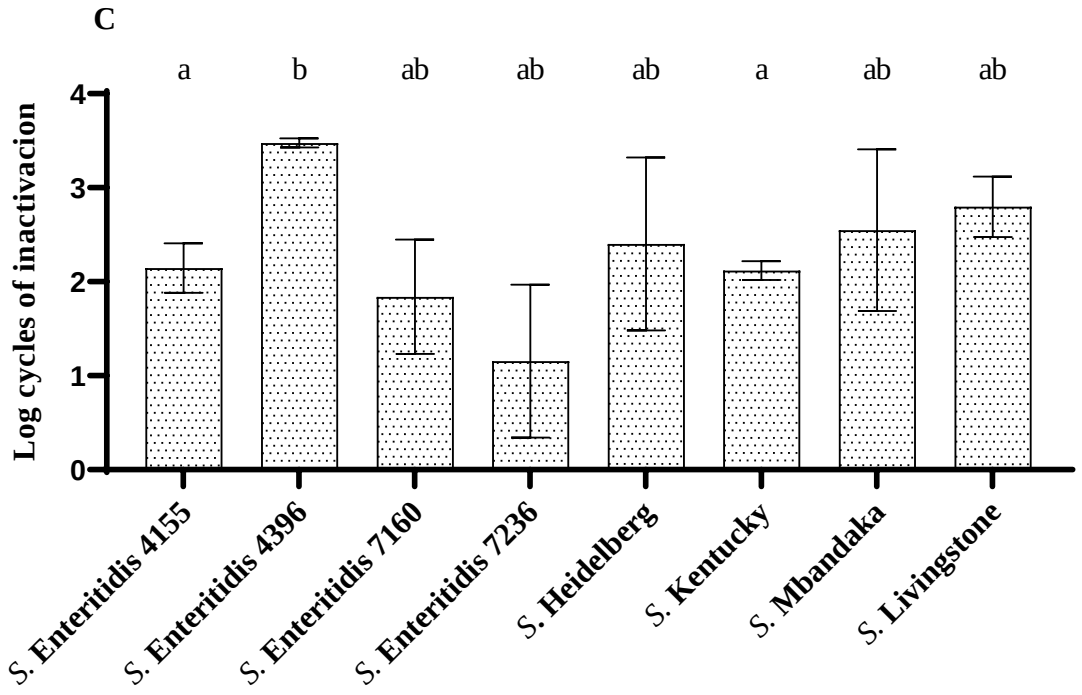

Figure 5. Resistance (Log cycles of inactivation) to different liquid egg decontamination/pasteurization technologies of 8 the *Salmonella* strains studied A) Heat treatment (2 min at 60 °C). B) PEF treatment (60 µs 23 kV/cm). C) HHP treatment (20 min at 300 MPa). Different letters indicate statistically significant differences between strains. Error bars correspond to the standard deviation of the means.

Table 1. Resistance (K_{max}. S_l and N_{res}) and goodness of the fit (R^2, *RMSE*) parameters calculated after fitting the survival curves to the 7 agents investigated of the 8 *Salmonella* strains studied to the Geeraerd's model.

	pH						***H_2O_2***						***NaCl***						***Heat***					
	***K_{max}* (min^{-1})**	***S_l* (min)**	***N_{res}* (CFU/ml)**	***2D*-value (min)**	***R^2***	***RMSE***	***K_{max}* (min^{-1})**	***S_l* (min)**	***N_{res}* (CFU/ml)**	***2D*-value (min)**	***R^2***	***RMSE***	***K_{max}* ($hour^{-1}$)**	***S_l* (hour)**	***N_{res}* (CFU/ml)**	***2D*-value (hour)**	***R^2***	***RMSE***	***K_{max}* (min^{-1})**	***S_l* (min)**	***N_{res}* (CFU/ml)**	***2D*-value (min)**	***R^2***	***RMSE***
***S.* Enteritidis 4155**	0.208 (0.012)	1.087 (1.883)	-	23.29 (0.639)	0.98-0.99	0.222 - 0.303	0.098 (0.002)	12.343 (3.729)	-	59.53 (3.041)	0.99 - 0.99	0.145 - 0.190	0.520 (0.048)	-	4.374 (0.034)	9.32 (0.944)	0.98 - 0.99	0.104 - 0.195	3.303 (0.398)	0.222 (0.197)	-	1.63 (0.02)	0.99 - 1.00	0.118 - 0.319
***S.* Enteritidis 4396**	0.128 (0.016)	-	-	36.29 (4.255)	0.97-0.99	0.146 - 0.228	0.111 (0.007)	19.097 (4.507)	-	60.61 (3.775)	0.97 - 0.98	0.237 - 0.353	0.512 (0.154)	-	3.719 (0.202)	8.22 (1.703)	0.95 - 0.96	0.291 - 0.302	2.951 (0.499)	0.053 (0.091)	-	1.64 (0.194)	0.95 - 1.00	0.120 - 0.557
***S.* Enteritidis 7160**	0.100 (0.003)	-	-	45.88 (1.240)	0.91-0.98	0.155 - 0.321	0.121 (0.005)	15.943 (3.566)	-	53.92 (4.384)	0.97 - 0.98	0.353 - 0.402	0.539 (0.033)	-	4.338 (0.036)	9.00 (0.645)	0.97 - 0.99	0.141 - 0.205	2.730 (0.292)	0.010 (0.012)	-	1.71 (0.181)	0.98 - 1.00	0.01 - 0.330
***S.* Enteritidis 7236**	0.113 (0.006)	-	-	40.72 (2.06)	0.86 - 0.88	0.428 - 0.508	0.142 (0.014)	24.560 (2.520)	-	57.27 (4.318)	0.99 - 1.00	0.118 - 0.197	0.547 (0.061)	-	4.556 (0.120)	9.18 (1.112)	0.95 - 0.99	0.106 - 0.260	2.093 (0.279)	0.040 (0.069)	-	2.26 (0.209)	0.96 - 1.00	0.124 - 0.472
***S.* Heidelberg**	0.174 (0.012)	1.055 (1.827)	-	27.69 (3.790)	0.99 - 1.00	0.084 - 0.234	0.135 (0.069)	17.953 (7.463)	-	57.72 (8.794)	0.98 - 1.00	0.134 - 0.295	1.306 (0.268)	-	3.204 (0.681)	3.66 (0.810)	0.98 - 1.00	0.090 - 0.360	1.874 (0.120)	0.237 (0.411)	-	2.70 (0.570)	0.97-0.99	0.171 - 0.335
***S.* Kentucky**	0.320 (0.029)	3.072 (4.995)	-	17.53 (4.050)	0.99 - 1.00	0.117 - 0.344	0.191 (0.059)	25.820 (12.920)	-	51.93 (3.369)	0.98 - 1.00	0.097 - 0.323	0.642 (0.093)	-	2.578 (0.390)	7.28 (0.988)	0.98 - 1.00	0.019 - 0.275	1.614 (0.646)	0.995 (1.416)	-	4.21 (0.865)	0.93 - 0.99	0.076 - 0.329
***S.* Mbandaka**	0.265 (0.078)	6.712 (5.913)	-	25.30 (0.344)	0.99 - 1.00	0.095 - 0.188	0.132 (0.025)	21.413 (10.249)	-	57.19 (6.144)	0.96 - 1.00	0.075 - 0.336	0.540 (0.084)	-	2.955 (0.383)	8.69 (1.197)	0.95 - 1.00	0.083 - 0.441	1.472 (0.280)	-	-	3.21 (0.659)	0.88 - 0.98	0.142 - 0.618
***S.* Livingstone**	0.195 (0.023)	2.148 (3.720)	-	26.04 (1.046)	0.98-0.99	0.181 - 0.253	0.103 (0.020)	15.238 (8.584)	-	61.17 (4.113)	0.98 - 1.00	0.091 - 0.213	0.539 (0.042)	-	3.832 (0.424)	8.72 (0.744)	0.98 - 0.99	0.167 - 0.208	1.231 (0.307)	-	-	3.89 (0.870)	0.90 - 0.97	0.252 - 0.322

* Values in parentheses represent the SD of the means.

Table 1. Continuation

	HHP						*PEF*						*UV-C*					
	K_{max} (min^{-1})	S_l (min)	N_{res} (CFU/ml)	*2D*-value (min)	R^2	*RMSE*	K_{max} (μs^{-1})	S_l (μs)	N_{res} (CFU/ml)	*2D*-value (μs)	R^2	*RMSE*	K_{max} (s^{-1})	S_l (s)	N_{res} (CFU/ml)	*2D*-value (s)	R^2	*RMSE*
***S.* Enteritidis 4155**	1.032 (0.060)	-	3.165 (0.075)	4.48 (0.261)	0.98 - 1.00	0.019 - 0.158	0.085 (0.004)	-	4.309 (0.048)	56.29 (2.343)	0.97 - 0.99	0.168 - 0.366	0.0581 (0.011)	7.727 (9.170)	-	65.39 (5.896)	0.98 - 1.00	0.009 - 0.337
***S.* Enteritidis 4396**	1.617 (0.027)	-	2.159 (0.158)	2.85 (0.047)	0.98 - 0.99	0.405 - 0.523	0.172 (0.005)	-	4.151 (0.086)	27.35 (0.910)	0.97 - 1.00	0.104 - 0.404	0.092 (0.006)	3.766 (3.454)	-	54.13 (4.082)	0.98 - 0.99	0.160 - 0.320
***S.* Enteritidis 7160**	0.501 (0.003)	-	-	9.19 (0.048)	0.90 - 0.91	0.475 - 0.517	0.087 (0.004)	-	4.356 (0.087)	55.61 (2.028)	0.97 - 0.99	0.237 - 0.366	0.074 (0.003)	5.986 (5.297)	-	68.63 (7.837)	0.93 - 0.99	0.179 - 0.655
***S.* Enteritidis 7236**	0.488 (0.010)	-	-	9.44 (0.196)	0.92 - 0.94	0.382 - 0.460	0.078 (0.001)	-	4.419 (0.120)	52.25 (1.276)	0.99 - 1.00	0.108 - 0.163	0.052 (0.001)	12.357 (0.835)	-	78.25 (0.876)	0.98 - 1.00	0.046 - 0.329
***S.* Heidelberg**	0.569 (0.052)	-	-	8.13 (0.724)	0.95 - 1.00	0.082 - 0.443	0.180 (0.035)	-	3.977 (0.626)	26.63 (5.101)	0.99 - 1.00	0.070 - 0.161	0.078 (0.011)	8.820 (12.473)	-	68.64 (4.045)	0.99 - 0.98	0.180 - 0.292
***S.* Kentucky**	0.837 (0.077)	0.493 (0.405)	-	5.86 (0.784)	0.96 - 1.00	0.225 - 0.408	0.185 (0.022)	-	4.133 (0.295)	25.78 (3.458)	0.99 - 1.00	0.018 - 0.189	0.073 (0.005)	13.560 (19.177)	-	76.44 (14.849)	0.99 - 1.00	0.091 - 0.165
***S.* Mbandaka**	1.178 (0.185)	-	3.632 (0.079)	4.02 (0.655)	0.93 - 1.00	0.089 - 0.543	0.178 (0.030)	-	4.032 (0.171)	26.60 (4.142)	0.95 - 0.99	0.203 - 0.600	0.084 (0.016)	32.180 (9.164)	-	87.93 (1.202)	0.99 - 1.00	0.098 - 0.176
***S.* Livingstone**	0.692 (0.077)	-	3.733 (0.176)	6.83 (0.810)	0.96 - 1.00	0.036 - 0.375	0.095 (0.006)	-	4.456 (0.215)	51.34 (2.644)	0.99 - 1.00	0.117 - 0.195	0.068 (0.023)	17.925 (12.482)	-	89.48 (11.730)	0.99 - 1.00	0.052 - 0.247

* Values in parentheses represent the SD of the means.

Table 2. Pearson correlation coefficient values calculated for the *2D* resistance values of the 8 *Salmonella* strains to the different environmental stresses and non-thermal food preservation technologies studied. Values in parentheses correspond to the *p*-value (p=0.05)

	pH	**H_2O_2**	**NaCl**	**Heat**	**HHP**	**PEF**	**UV**
pH		0.013 (0.976)	0.480 (0.276)	-0.661 (0.075)	0.481 (0.228)	0.476 (0.233)	-0.314 (0.449)
H_2O_2	0.013 (0.976)		0.452 (0.309)	-0.264 (0.528)	-0.363 (0.377)	0.154 (0.716)	-0.089 (0.834)
NaCl	0.480 (0.276)	0.452 (0.309)		-0.606 (0.149)	0.325 (0.477)	0.792 (0.034)	0.028 (0.952)
Heat	-0.661 (0.075)	-0.264 (0.528)	-0.606 (0.149)		0.010 (0.981)	-0.374 (0.361)	0.737 (0.037)
HHP	0.481 (0.228)	-0.363 (0.377)	0.325 (0.477)	0.010 (0.981)		0.526 (0.181)	0.234 (0.577)
PEF	0.476 (0.233)	0.154 (0.716)	0.792 (0.034)	-0.374 (0.361)	0.526 (0.181)		0.113 (0.789)
UV	-0.314 (0.449)	-0.089 (0.834)	0.028 (0.952)	0.737 (0.037)	0.234 (0.577)	0.113 (0.789)	

HIGHLIGHTS

Stress resistance of 4 poultry-related *Salmonella* serovars and *S.* Enteritidis was compared.

A good correspondence between the results obtained in buffer and food matrixes was observed.

Emerging serovars displayed a lower acid and osmotic stress resistance than S. Enteritidis.

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