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# Modeling the combined resistance to microwave treatments and salt conditions of *Escherichia coli* and *Staphylococcus aureus*

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## Abstract

In the present study, the efficiency of the combined effect of microwave irradiation treatments together with salt concentration was assessed against *Escherichia coli* and *Staphylococcus aureus*. Microbial survival has been modeled through a one-step Weibull equation considering the non-isothermal profiles during the heating treatments. Three sodium chloride concentrations 0.5%, 3.5%, and 8.5% (*w*/*v*) treated under three microwave power levels (450, 600, and 800 W) were studied. Predictive models were validated using the determination coefficient ($R^2$), root mean squared error and the acceptable prediction zone with external data obtained from ultra high temperature milk. The results obtained suggested that increasing microwave power levels and decreasing salt concentrations led to a higher microbial inactivation, being the $\delta$ values (time for achieving a first decimal reduction) for *E coli* of 19.57 s at 800 W and 0.5% NaCl. In contrast, experimental data of *S aureus* showed a higher variability since it presented more resistance to the microwave treatments. The results obtained and generated models can be used as decision-making tools to set specific guidelines on microwave treatments for assuring food safety.

## Keywords

Dynamic inactivation, *Escherichia coli*, hurdle technology, *Staphylococcus aureus*, Weibull models

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## INTRODUCTION

Nowadays, microwave irradiation is widely used due to advances in device consistency, minimization of undesirable disinfection-by products, and household applications (Shaw et al., 2021). Additionally, microwave usage in industrial applications, notably in the food and associated industries, has grown in popularity (Gedikli et al., 2008).

Microwave irradiation has been traditionally used to inactivate a wide range of microorganisms including pathogenic vegetative bacteria, bacteria forming endospores, vegetative spoilage bacteria, parasites, fungi, and bacteriophages (Bozkurt-Cekmer and Davidson, 2017).

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*Escherichia coli* and *Staphylococcus aureus* are ubiquitous organisms that may be found on humans and animals. They are also opportunistic pathogenic bacteria that can cause human illnesses. The essential advantage of using microwave energy has been the ability to sterilize at lower temperatures and in a shorter time than is required by conventional heating procedures (Fung and Cunningham, 1980). It is usually assumed that microbial inactivation is mostly induced by an increase in temperature following microwave exposure (Rougier et al., 2014). As microwave power and time are increased, the treatment's efficacy improves as shown in other studies (Valero et al., 2014).

According to Shamis et al. (2012) microwaves are electromagnetic waves that have frequencies ranging from 0.3 to 300 GHz and wavelengths that are between 1 m and 1 mm. They are types of non-ionizing radiation that are present close to the infrared spectrum (Wang et al., 2019). The heat produced is a consequence of the microwave energy being absorbed by dielectric substance, mainly water and being converted to heat by the rotation's internal resistance (Heddleson and Doores, 1994; Kozempel et al., 2000). In other words, the frictional forces arising from the movement of dipolar molecules dissipate energy in the form of heat (Shamis et al., 2012).

Regarding the use of microwave treatments to inactivate microorganisms in foods, there is a number of factors, including food moisture and ion content, the specific heat of the various components of the food, the density of the product, its shape and the volume of the load, which impact microwave energy absorption and modulate the efficiency of the treatment (Campanone and Zaritzky, 2005; Hossan and Dutta, 2012).

The dissolved salt content decreases the depth of penetration of microwave energy, thus creating a decreasing temperature gradient from the surface, then the microwave heating results in a non-uniform temperature distribution within the product (Goksoy et al., 1999; Ramaswamy and Pillet-Will, 1992; Vadivambal and Jayas, 2010). This is one of the primary downsides of microwave heating and it might result in partial inactivation by keeping bacteria alive in the less heated places (Apostolou et al., 2005; Heddleson et al., 1996).

Predictive microbiology models describe and quantify the behavior of foodborne pathogens and spoilage microorganisms in foods under different environmental conditions. Predictive models are recognized as useful tools to assess food safety and quality risks and are used as decision-making tools in both regulatory agencies and food industries (Perez-Rodriguez and Valero, 2013). To describe the survival of microorganisms, the Weibull model has been widely applied given its flexibility ease of application in food commodities (van Boekel, 2002).

The main objective of this study was to assess and determine the effect of microwaves and the presence of sodium chloride on the inactivation kinetics of *E coli* and *S aureus*. To achieve this, the procedure followed was first describing the inactivation kinetics of *E coli* and *S aureus* under different microwave powers at different concentrations of sodium chloride. Then inactivation models were developed for the first time to the best of our knowledge to predict microbial survival under dynamic conditions and subsequently the influence of NaCl on the microwave inactivation kinetics was discussed. Finally, a model validation was carried out in UHT milk.

## MATERIAL AND METHODS

### Bacterial cultures

*E coli* and *S aureus* starins were obtained from the Spanish Type Culture Collection (CECT, Burjassot, Valencia). Both strains were kept at −80 °C in cryovials that included beads and cryopreservatives.

To reconstitute the strains, one bead of each bacterial strain was put into a tube containing 9 mL of Tryptone Soy Broth (TSB, Oxoid, UK) three days before the test and incubated at 37 °C for 24 h. For each strain, a second subculture was prepared by inoculating tubes containing 9 mL of TSB with 0.1 mL of the initial subcultures and incubating under the same conditions (37 °C for 24 h). Finally, a third subculture of each bacterial suspension was prepared and incubated at 37 °C for 14 h, as described before to reach the early stationary phase. The final fresh subcultures obtained were centrifuged for 10 min at 4000 r/min. The precipitates were resuspended in 10 mL of phosphate-buffered saline (PBS, Oxoid). A microbiological count was done to standardize the concentration of the inoculum at a concentration of approximately $10^8$–$10^9$ CFU/mL. A calibration of the inoculum concentration was obtained by microbial counts on agar medium in Petri dishes. The number of bacteria obtained is expressed in colony-forming unit (CFU)/mL.

### Preparation of media and addition of salt solutions

The study was performed on buffered peptone water (BPW, the NaCl content is 5 g/L and pH is $7.2 \pm 0.2$ at 25 °C). Sodium chloride was added to the BPW (Oxoid) at concentrations of 3.5% and 8.5% (*w/v*). Glass flasks of 500 mL and 86 mm diameter were used to put 50 mL of BPW. The flasks were sterilized by autoclaving and inoculated with 1 mL of the reconstituted suspensions of *E coli* and *S aureus*, previously prepared. After inoculation, the flasks were placed in the geometric center of a household microwave oven and submitted to microwave irradiation.

### Microwave treatments

The microwave treatments were achieved in a commercial microwave oven with a frequency of 2450 MHz and a maximum exit power of 800 W (Samsung M1727,

Korea). The treatments of all solutions were processed at three power levels: 450, 600, and 800 W. According to the concentrations of the treated solutions, the irradiation times was adjusted at 70, 50 and, 40 s for 450, 600 and, 800 W, respectively when the BPW was at 0.5% of NaCl. At 3.5% and 8.5% NaCl concentrations, the microwave treatment times were 100 s (450 W), 70 s (600 W), and 50 s (800 W). The levels of the variables studied were defined in primary assay according to a thermal history, so that, for each selected power, a temperature measurement was carried out every 5 s until a maximum required temperature was reached (70 and 80 °C). This choice was made because the lethal temperatures of *E coli* and *S aureus* are 70 and 65 °C, respectively.

The temperatures were measured using a food thermometer equipped with a specialized probe (HI 95809–1, Hanna instruments). The temperatures were taken from the middle of the irradiated solutions after mixing.

Following each time interval treatment, the flasks were immediately placed in ice-containers for cooling before being subjected to microbiological analysis.

## MICROBIAL ANALYSES

After each microwave treatment, serial dilutions were done in saline solution (0.9%, w/v) and plated on PCA agar (Oxoid), and then incubated for 24 h at 37 °C. After incubation, colonies were enumerated using an automatic colony counter Flash & Go (IUL Instruments, Barcelona, Spain).

### Modeling of survival curves

For modeling purposes, the residual population density (log $N_{res}$) was considered as 0.1 log CFU/mL when no colonies were detected. To model the survival of *E coli* and *S aureus* as a function of the microwave intensity and salt concentrations, a Weibull model was chosen. First, the classical two-step procedure was followed. This was followed by a one-step fitting to consider the effect of non-isothermal treatments.

On the first part of the analysis, survival models were built as a function of the microwave power level (800, 600, and 450 W) applied and salt concentrations (0.5, 3.5, and 8.5%). Fitting was performed using R 4.2.1 (R Core Team, 2020) and R package "nls Microbio" (Baty and Delignette-Muller, 2017).

The model that describes destruction profiles without tails uses equation 1 (Mafart et al., 2002), and the model that fits destruction profiles characterized by a tail uses equation 2 (Albert and Mafart, 2005) are:

$$\text{Log } S_{(t)} = \left[\frac{t}{\delta}\right]^{p} \tag{1}$$

$$S_{(t)} = (N_0 - N_{res})10^{\left[-\left(\frac{t}{\delta}\right)^{p}\right]} + N_{res} \tag{2}$$

The estimated parameters corresponded to the time for the first decimal reduction ($\delta$, [sec]) and the shape parameter ($p$, [-]) (van Boekel, 2002). $N_0$and $N_{res}$ are the initial and residual microbial populations (CFU/mL), being $S_{(t)}$ the survival population of each microorganism at time t.

For the secondary modeling, the heating rates (*hr*, [°C/s]) were estimated through the slope of the regressions of temperature and time, at each microwave treatment. The effect of temperature on the Weibull model parameter, $\delta$, was modeled by this equation:

$$\log \delta = a * hr + b \tag{3}$$

where $a$ and $b$ are the coefficients to be estimated and *hr* is the heating rate (°C/s). When necessary, the natural logarithm (*ln*) was applied to the first term of the equation to improve the fitting of the model.

Finally, a global Weibull model was generated through a one-step approach by combining the primary and secondary models and setting a fixed value of $p$ for each salt concentration. To check potential differences in the model fitting, the individual values of $p$ were compared using $F$-test and that value presenting the best fit was selected for each salt concentration and microorganism.

The determination coefficient $R^2$, and root mean squared error (RMSE) were used to evaluate the performance of the predictive models in adequately describing the observed experimental data. $R^2$ was utilized as an overall evaluation of the precision of the prediction.

### Comparison of inactivation kinetics

To enable a direct comparison of the inactivation kinetics, the time required to obtain 1, 2, 3 and 4 log reduction levels ($t_x$, [s]) was calculated (Buchanan et al., 1993) based on the fitted parameters of the global Weibull models. To estimate $t_x$, the following equation was applied:

$$t_x = \delta * (x)^{1/p} \tag{4}$$

Normal distribution and homogeneity of variances were tested using Shapiro-Wilk test and Levene test, respectively.

## MODEL VALIDATION

The generated models in broth culture were validated using independent data obtained in UHT milk. Two microwave treatments were tested, 800 and 600 W.

Accordingly, 50 mL of milk in 500 mL glass flasks were inoculated with 1 mL of the bacterial suspension of each bacterial strain (*E coli* and *S aureus*) to reach a concentration of $10^6$–$10^7$ CFU/mL. Microwave heating was tested at two power levels (600 W-40 s and 800 W-30 s). Prior to microbiological analysis, the samples were put in an ice bath at each interval to stop the residual heating. After

microwave irradiation, 1 mL aliquots were serially diluted with 9 mL of physiological water, and then 1 mL was inoculated on PCA Agar (Oxoid). All plates were incubated at 37 °C for 24 h. After incubation, cell counting was realized with colony counter the Flash & Go.

To assess model validation, the acceptable prediction zone (APZ) has been used as recommended by Oscar (Oscar, 2005).This index estimates the percentage of residuals within the −1 to 0.5 log CFU/mL. Further, the validation indices, the accuracy factor ($A_f$) and bias factor ($B_f$) proposed by Ross (1996) have been calculated for the *E coli* and *S aureus*. The $A_f$ quantifies the spread of observed data relative to model predictions, while the $B_f$ measures the degree to which the model under- or over estimates the observed growth. $A_f$ and $B_f$ values closer to 1 indicate a better agreement between the experimental data and the model predictions.

## STATISTICAL ANALYSIS

The results are presented as the mean ± SD (standard deviation) of three independent experiments. Statistical analysis was performed with SPSS. Statistical tests were used to obtain the $p$-value was analysis of variance (ANOVA) followed by Tukey's HSD test. Differences at $p < 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

### Modeling of survival curves

Survival curves of *E coli* and *S aureus* at the different treatments were modeled through Weibull equations, considering the dynamic temperature profile of microwave treatments. The estimated parameters together with the goodness of fit indices are represented in Table 1. Figures 1 and 2 show the fitted Weibull models to describe the non-isothermal survival curves of *E coli* and *S aureus*. The survival curves for both microorganisms were convexly shaped, with a shoulder region preceding an acceleration of the decay rate, with or without a tailing effect.

The results showed that the $\delta$ values decreased with increasing microwave powers while the $p$-value varied according to the degree of convexity of each survival curve. This was noted by several authors in previous studies (Hassani et al., 2005; Kernou et al., 2021; van Boekel, 2002; Zhang et al., 2018). Thus, in order to limit the number of parameter values in the equation as much as possible, the survival curve was refitted taken into account a constant value of $p$. The $p$ value chosen was estimated from the best fit of the experimental values obtained at different concentrations of sodium chloride for each studied power level. However, $\delta$ values varied according to the microwave power and the concentration of NaCl. Thus, secondary models describing the linear relationship between the $\delta$ values and the heating rates ($hr$) have been developed as follows:

$$E.\ coli\ 0.5\%\ \text{NaCl}:\ \log\delta = -0.337\ (\pm 0.116) * \ln(hr) + 1.395\ (\pm 0.023) \quad (5)$$

$$E.\ coli\ 3.5\%\ \text{NaCl}:\ \log\delta = -0.360\ (\pm 0.078) * hr + 1.769\ (\pm 0.072) \quad (6)$$

$$E.\ coli\ 8.5\%\ \text{NaCl}:\ \log\delta = -0.114\ (\pm 0.038) * \ln(hr) + 1.573\ (\pm 0.018) \quad (7)$$

$$S.\ aureus\ 0.5\%\ \text{NaCl}:\ \log\delta = -0.515\ (\pm 0.058) * \ln(hr) + 1.493\ (\pm 0.011) \quad (8)$$

$$S.\ aureus\ 3.5\%\ \text{NaCl}:\ \log\delta = -0.423\ (\pm 0.050) * hr + 1.976\ (\pm 0.046) \quad (9)$$

$$S.\ aureus\ 8.5\%\ \text{NaCl}:\ \log\delta = -0.487\ (\pm 0.013) * hr + 2.009\ (\pm 0.010) \quad (10)$$

A one-step approach was followed by introducing the equations 5–10 into the Weibull primary model in order to predict bacterial inactivation under varying powers and salt concentrations.

For the shape $p$ parameter of the Weibull model, no secondary model could be developed. Therefore, the equation for the one-step modeling used the overall mean $p$ parameter estimates. In contrast to the secondary models a temperature-dependency of the log transformed parameter $\delta$ was captured by the model generated in the one-step fitting.

The determination coefficients ($R^2$ values) for *E coli* varied from 0.917 to 0.983. In the case of *S aureus*, $R^2$ values varied from 0.908 to 0.983 (Table 1). And the *RMSE* calculated from the models were ranged from 0.074 to 0.501 and from 0.154 to 0.842 for *E coli* and *S aureus,* respectively. The Weibull models described accurately the complete survival curves profiles of *E coli* and *S aureus* obtained, demonstrating their applicability as reported in other studies (Greenacre et al., 2003; Valdramidis et al., 2005).

### Influence of NaCl concentration on microbial inactivation during microwave treatment

The effect of NaCl on the effectiveness of microwave treatments on *E coli* and *S aureus* was observed on the time to require a certain reduction level ($t_x$, s), as shown in Table 2. The $t_x$ values changed depending on the microwave power and the NaCl concentration, such as the applied power increases, the $t_x$ decreases for both bacterial strains. This means an acceleration of the bacterial destruction by the increase of the applied microwave powers. On the contrary,

**Table 1.** Secondary models and δ values estimated of *E coli* and *S aureus* obtained under microwave treatments at 0.5%, 3.5% and 8.5% of NaCl.

| Microorganisms | NaCl | MW power | Hr (°C/s) | p | δ | $R^2$ | Root mean squared error (RMSE) |
|---|---|---|---|---|---|---|---|
| *E coli* | 0.5% | 800 | 1.344 | | 19.573 | 0.911 | 0.274 |
| | | 600 | 1.076 | 1.830 | 23.487 | 0.869 | 0.327 |
| | | 450 | 0.860 | | 28.222 | 0.932 | 0.251 |
| | 3.5% | 800 | 1.180 | | 22.147 | 0.844 | 0.501 |
| | | 600 | 0.902 | 2.016 | 27.756 | 0.960 | 0.176 |
| | | 450 | 0.600 | | 35.472 | 0.980 | 0.074 |
| | 8.5% | 800 | 1.066 | | 36.772 | 0.843 | 0.468 |
| | | 600 | 0.752 | 2.091 | 40.299 | 0.823 | 0.460 |
| | | 450 | 0.470 | | 45.590 | 0.963 | 0.147 |
| *S aureus* | 0.5% | 800 | 1.34 | | 21.764 | 0.809 | 0.392 |
| | | 600 | 1.08 | 13.90 | 28.332 | 0.851 | 0.293 |
| | | 450 | 0.86 | | 36.955 | 0.934 | 0.158 |
| | 3.5% | 800 | 1.18 | | 29.981 | 0.865 | 0.292 |
| | | 600 | 0.90 | 2.274 | 39.306 | 0.832 | 0.433 |
| | | 450 | 0.60 | | 52.752 | 0.910 | 0.261 |
| | 8.5% | 800 | 1.07 | | 30.870 | 0.769 | 0.842 |
| | | 600 | 0.75 | 2.157 | 43.911 | 0.841 | 0.154 |
| | | 450 | 0.47 | | 60.259 | 0.960 | 0.486 |

increasing the concentration of NaCl induced an increase of $t_x$ which resulted in a slowing down of the bacterial inactivation. This could be explained by the presence of NaCl which would offer a protection of the microorganisms against the temperatures generated by the different microwave powers applied (Heddleson et al., 1993).

Ionic compounds, such as sodium chloride (NaCl), are recognized to be responsible for increased temperature rise and cell inactivation. Ionic solutions have higher electroconductivity and electric properties that are significantly closer to those of conductors than pure water (Watanabe et al., 2000). When ionic salts are dissolved in water, the relaxation time falls at low concentrations and gradually increases, affecting the structure of the water. It has been suggested that the ions' presence leads water to undergo a structural breakdown process. The water molecules that are coordinated to the ions are rotationally locked; however, the uncoordinated water molecules do not suffer as strong intermolecular hydrogen bonding effects, and as a result, they have shorter relaxation durations in this more free state. The effects are reversed at higher concentrations, and the relaxation frequency of water in concentrated salt solutions is higher than that of pure water. This is probably because the water molecules are more ordered in the presence of many ions (Grant and Halstead, 1998).

Significant differences ($p < 0.05$) were obtained between power levels and NaCl concentrations for each microorganism. The highest $t_x$ values corresponded to 8.5% and 450 W treatments, ranging from 45.59 to 88.48 s for *E coli* and from 60.26 to 114.59 s for *S aureus* (Table 2). It was also shown that *S aureus* was more resistant to the applied treatments than *E coli* regardless the NaCl concentration and power level ($p < 0.05$). Then, it seems that using moderate heat powers and increasing NaCl concentrations may induce a protection for the microorganisms against heat (Heddleson et al., 1993).

Several researches have previously studied the influence of salinity on microwave bacterial treatment efficacy. Dealler and Lacey (1990) prepared mashed potato mixed with various concentrations of ionic compounds (NaCl, KCl, $NH_4Cl$, and sodium glutamate) and was irradiated under microwaves at 1250-MHz, they discovered that increasing concentrations of the ionic compounds added suppressed the temperature increase at the core part. When an *E coli* cell solution was microwave-irradiated in a glass beaker containing various concentrations of NaCl, Fujikawa et al. (1992) observed that increasing the NaCl content inhibited bacterial death. Heddleson et al. (1993) investigated the relationship between the quantity of NaCl present in saline solutions, the temperatures that were reached, and the level to which *Salmonella* spp. might be inactivated. They reported that sodium chloride serves a dual effect in determining their survival by producing a thermal stress of microbial cells and a temperature change in the broth media. This suggests that sodium chloride plays a role in both aspects of this process. They also observed that salts in solutions, when present in appropriate quantities, may cause a reduction in the penetration depth of microwaves. This will result in more energy being absorbed closer to the surface of the solution (Heddleson et al., 1993). Morozov et al. (2017) used different concentrations of NaCl to demonstrate the protection of bacteria from the thermal damage caused by SHF (super high frequency: frequencies fall within the microwave band) at 50 and 60 °C. The

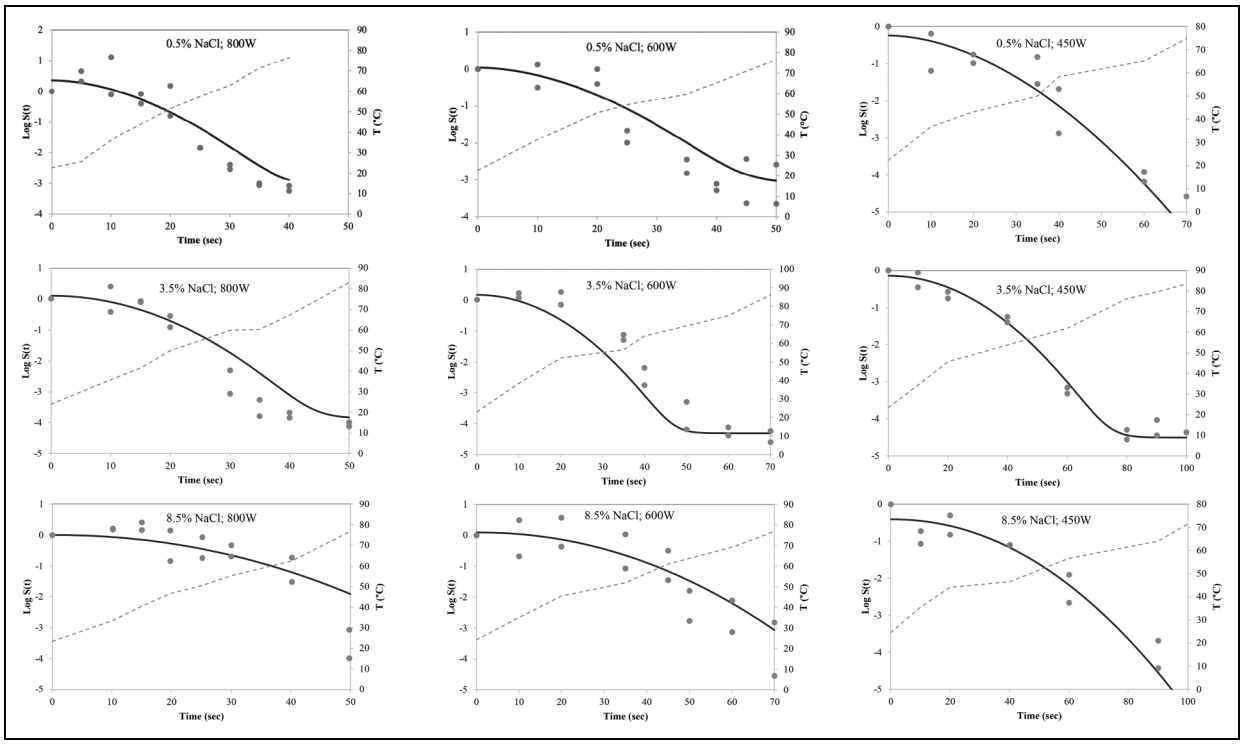


**Figure 1.** Fitting of the Weibull modified models (solid lines) to the observed survival data (log $S_{(t)}$) (symbols) of *E coli* subjected to microwave treatments. Dashed lines represent the evolution of temperature (° C) during the microwave treatments.

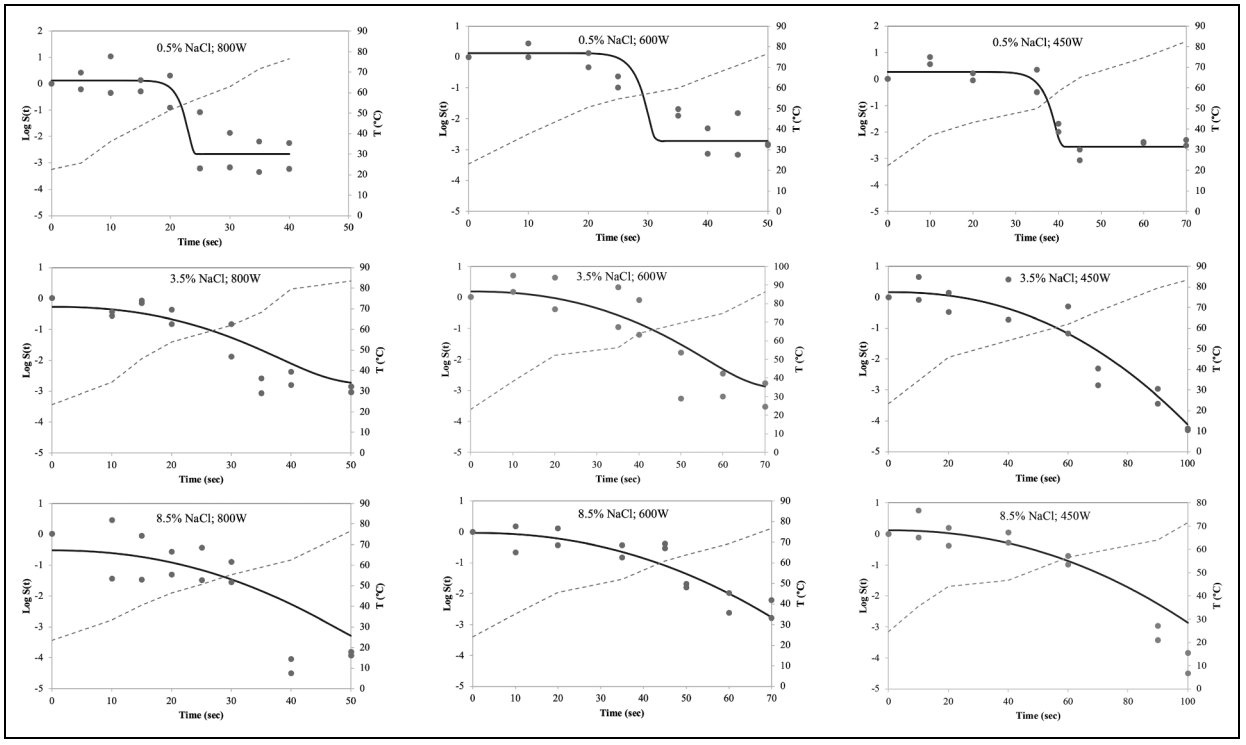


**Figure 2.** Fitting of the Weibull modified models (solid lines) to the observed survival data (log $S_{(t)}$) (symbols) of *S. aureus* subjected to microwave treatments. Dashed lines represent the evolution of temperature (°C) during the microwave treatments.

**Table 2.** Predicted values for the time to achieve a 1, 2, 3, and 4 logarithmic reductions of *E coli* and *S aureus* at the different microwave treatments and NaCl concentrations.

| Microorganism | Power level | NaCl% | $t_{1D}$ (s) | $t_{2D}$ (s) | $t_{3D}$ (s) | $t_{4D}$ (s) |
|---|---|---|---|---|---|---|
| *E coli* | 800 W | 0.5 | 19.57 | 28.58 | 35.67 | 41.75 |
| | | 3.5 | 22.15 | 31.23 | 38.19 | 44.05 |
| | | 8.5 | 36.77 | 51.23 | 62.19 | 71.36 |
| | 600 W | 0.5 | 23.49 | 34.30 | 42.81 | 50.09 |
| | | 3.5 | 27.76 | 39.14 | 47.86 | 55.20 |
| | | 8.5 | 40.30 | 56.14 | 68.15 | 78.21 |
| | 450 W | 0.5 | 28.22 | 41.22 | 51.44 | 60.19 |
| | | 3.5 | 35.47 | 50.02 | 61.17 | 70.55 |
| | | 8.5 | 45.59 | 63.51 | 77.10 | 88.48 |
| *S aureus* | 800 W | 0.5 | 21.76 | 23.01 | 23.70 | 24.19 |
| | | 3.5 | 29.98 | 40.66 | 48.60 | 55.15 |
| | | 8.5 | 30.87 | 42.57 | 51.37 | 58.70 |
| | 600 W | 0.5 | 28.33 | 29.96 | 30.85 | 31.49 |
| | | 3.5 | 39.31 | 53.31 | 63.72 | 72.31 |
| | | 8.5 | 43.91 | 60.55 | 73.07 | 83.50 |
| | 450 W | 0.5 | 36.95 | 39.08 | 40.24 | 41.08 |
| | | 3.5 | 52.75 | 71.55 | 85.51 | 97.04 |
| | | 8.5 | 60.26 | 83.10 | 100.28 | 114.59 |
| *E coli* (ultra high temperature (UHT) milk) | 800 W | 0.5 | 14.63 | 21.36 | 26.66 | 31.20 |
| | 600 W | 0.5 | 18.95 | 26.72 | 32.67 | 37.69 |
| *S aureus* (UHT milk) | 800 W | 0.5 | 14.37 | 15.10 | 15.55 | 15.88 |
| | 600 W | 0.5 | 20.89 | 28.34 | 33.87 | 38.43 |

results demonstrated a clear effect of cell defense against damage. At different temperatures, the efficiency of the protection against thermal damage produced by NaCl relies on the degree of the heat exposure, being the highest resistance obtained at 60 °C and 7.5% NaCl. However, according to Watanabe et al. (2000) the increasing concentration of ionic compounds in the cell suspension produced a higher microbial inactivation. They explained this by suggesting that the form of the container may alter the heating intensity caused by microwave irradiation.

## MODEL VALIDATION

The Weibull models have been validated using UHT milk at two power levels (800 and, 600 W) for *E coli* and *S aureus*. The fitted survival curves are represented in Figures 3 and 4. It can be seen that for *E coli* there was a slight decay over time until reaching a reduction of ca. 3 log CFU/mL at 800 and 600 W. In contrast, *S aureus* population remained within 1 log reduction during the first 15 s treatment, showing a linear decay afterwards, being the total reduction ca. 4 log CFU/mL for 800 and 600 W treatments.

To accurately assess the goodness of fit of the Weibull models, the APZ was calculated by the differences between predicted and observed values. The graphical representations are shown in Figures 5 and 6. It was seen that for *E coli*, 84.30% of the data fell inside the APZ (+1 to −0.5 log CFU/mL), while more variability was observed for *S aureus* since 72.41% were within the APZ. This variability can be associated to the increased

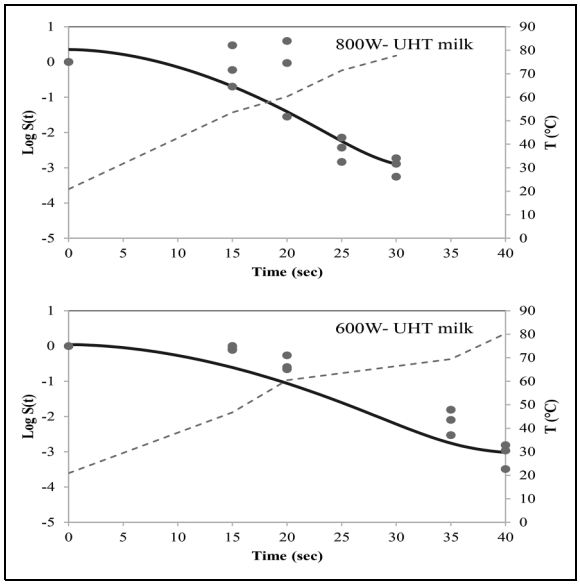


**Figure 3.** Validation of the Weibull modified models (solid lines) to the observed survival data (log $S_{(t)}$) (symbols) of *E coli* subjected to microwave treatments in ultra high temperature (UHT) milk at 800 and 600 W. Dashed lines represent the evolution of temperature (°C) during the microwave treatments.

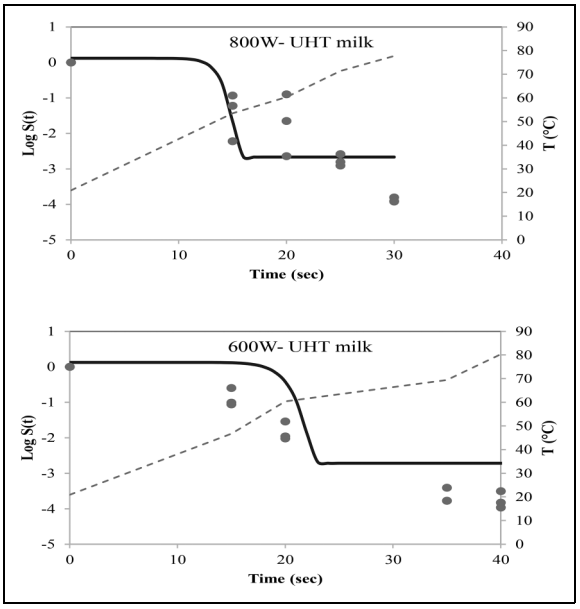


**Figure 4.** Validation of the Weibull modified models (solid lines) to the observed survival data (log $S_{(t)}$) (symbols) of *S aureus* subjected to microwave treatments in ultra high temperature (UHT) milk at 800 and 600 W. Dashed lines represent the evolution of temperature (°C) during the microwave treatments.

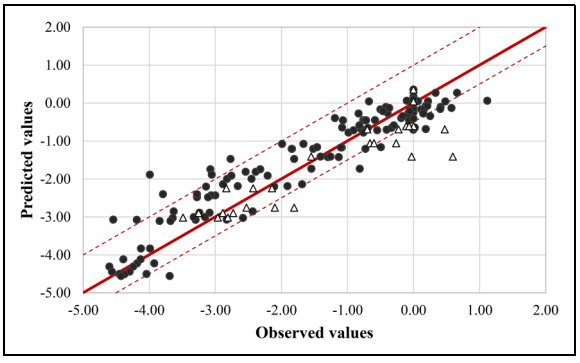


**Figure 5.** Graphical representation of observed vs predicted values for model data (black circles) and validation data in ultra high temperature (UHT) milk (white triangles) for *E coli* at the different microwave treatments. The equivalence line is represented by the solid red line, while the dotted lines define the acceptable prediction zone (+1 to −0.5 log CFU/mL).

resistance of *S aureus* to the different microwave treatments and NaCl applied. However, it was considered that in both cases, the Weibull models provided a reasonable description of the observed data.

As additional validation indices, the $A_f$ and $B_f$ values were calculated to assess models' performance. Regarding *E coli*, $B_f = 1.033$ and $A_f = 1.195$, showing a slight overestimation of microbial inactivation. For *S aureus* $B_f = 0.925$ and $A_f = 1.104$ so that it was evidenced an under-estimation

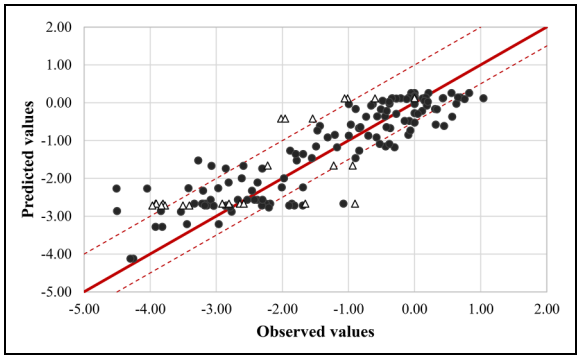


**Figure 6.** Graphical representation of observed vs predicted values for model data (black circles) and validation data in ultra high temperature (UHT) milk (white triangles) for *S aureus* at the different microwave treatments. The equivalence line is represented by the solid red line, while the dotted lines define the acceptable prediction zone (+1 to −0.5 log CFU/mL).

of microbial reductions, which can be seen at 600 W (Figure 4).

However, the model was validated in a matrix of different nature as the culture medium, such as UHT milk. As it is presented in a form of emulsion, the physical properties and the different nutritional composition would lead to limit the applicability of the model. However, as shown with the obtained validation indices, the model can be effectively applied for this matrix to predict microbial fate at different salt concentrations and microwave treatments.

## CONCLUSIONS

The determination of the influence of microwaves and the presence of sodium chloride on the inactivation kinetics of *E coli* and *S aureus* was investigated. Many environmental conditions have an essential effect in assessing the dielectric characteristics of meals. Peptone-buffered water was used as a model medium to eliminate the influence of external variables. Bacterial destruction was performed at three NaCl concentrations of 0.5%, 3.5%, and 8.5% (*w/v*) and three microwave powers (450, 600 and, 800 W). The results indicated that rising microwave power levels (linked to the increasing of the irradiation time) and lowering salt concentrations resulted in higher microbial inactivation, with the δ values time for reaching a first decimal reduction) for *E coli* at 800 W and 0.5% NaCl being 19.57 s As comparison, *S aureus* experimental results exhibited greater variability because it was more resistant to microwave treatments. The obtained results and the generated patterns can be applied as tools for decision making in order to establish specific instructions on microwave treatments to ensure the food security.

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