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High hydrostatic pressure inactivation of microorganisms: A probabilistic model for target log-reductions

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ABSTRACT

A probabilistic model based on logistic regression was developed for a target log reduction of microorganisms inactivated by high hydrostatic pressure. Published inactivation data of *Salmonella* Typhimurium in broth for 4 and 5 log reductions, and *Escherichia coli* in buffer and carrot juice for 5 log reduction were used. The probabilities of achieving 4 or 5 log reductions for *S.* Typhimurium in broth and 5 log reduction for *E. coli* in buffer and carrot juice could be calculated at different pressure, temperature and time levels. The fitted interfaces of achieving/not achieving the target log reduction were consistent with the experimental data. Although the reliability of the predictions of the developed models could be questioned due to strain variation and different food matrix, a validation study has demonstrated that the developed models could be used to predict the target log reduction of these microorganisms at different pressure, temperature and time levels. This study has indicated that the probabilistic modeling for target log reductions can be useful tool for HHP inactivation of microorganisms, but further studies could be performed with several other factors such as pH and water activity of the food, concentration of certain additives as well as initial number of bacteria present in the food.

1. Introduction

High hydrostatic pressure (HHP) treatment has been applied to certain foods for more than two decades and it is now well-known worldwide (Buzrul, 2014). One of the most important properties of the HHP treatment is the ability to inactivate different types of microorganisms in different types of foods (Simpson and Gilmour, 1997). This is well documented in literature. For example, foodborne pathogens in milk (De Lamo-Castellví et al., 2005; Mussa et al., 1999; Solomon and Hoover, 2004) and meat (Hereu et al., 2012; Morales et al., 2009), spoilage bacteria and yeasts in fruit juices (Basak et al., 2002; Donsì et al., 2007) can be successfully inactivated by HHP treatment. Despite the commercial use of HHP to destroy microorganisms in foods, several concerns such as the effect of different food matrix on inactivation post pressure survival or injury recovery and strain variability to HHP still exist (Gänzle and Liu, 2015): Foods with low water activity have been shown to be challenging for microbial inactivation by HHP (Georget et al., 2015). Cells, that are undetectable after HHP treatment, can recover from injury during post-pressure storage (Koseki and Yamamoto, 2006). A large variability in resistance to HHP, not only among the species but also among the strains of the same species, can be observed (Liu et al., 2015). Some strains can resist even pressures up to 600 MPa at ambient temperature (Tassou et al., 2008). Therefore, safe elimination of bacteria in food by HHP are dependent on many intrinsic and extrinsic factors.

The cost of HHP processing mainly depends on the target (final) pressure, processing temperature and holding time. In order to optimize processing parameters of HHP treatment, modeling studies are needed. Although dose-response modeling has been recently demonstrated to be successful for HHP inactivation of microorganisms (Buzrul, 2017), mostly kinetic modeling studies were conducted. In other words, log inactivation versus time data at certain pressure and temperature levels were plotted and suitable model or models were fitted and further model validation were performed (Buzrul and Alpas, 2004; Buzrul et al., 2005; Chen and Hoover, 2003, 2004).

Beside kinetic modeling, probabilistic modeling could also be used. In this modeling technique binary (dichotomous) response such as growth/no growth or survival/death is defined and modeling is applied by logistic regression procedure. This type of modeling in the predictive microbiology was mainly used to determine growth interface of different microorganisms (McKellar and Lu, 2001; Tienungoon et al., 2000); however, application of probabilistic models for HHP inactivation of microorganisms are scarce. Two notable examples are the works of Koseki and Yamamoto (2007) and Koseki et al. (2009). In these studies, survival/death interface of *Listeria monocytogenes* and *Cronobacter* spp. (*Enterobacter sakazakii*) were defined under different

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combinations of factors such as pressure, temperature, time, pH and inoculum level.

Commercial HHP treatments require shorter (< 10 min) holding times since the cost of HHP processing increases with the use of long holding times (Buzrul, 2017). Probabilistic models can be used in this manner: holding time values < 10 min can be selected for the modeling purposes and whole experimental design can be set up according to this selection. If the target log reduction is definite (5 or 6 log reductions for pasteurization) for the food that will be processed, pressure and temperature values can be chosen for this definite target log reduction of specific microorganisms (spoilage or pathogenic) and probabilistic modeling can be applied.

Recently, logistic regression models were used for the growth of *Staphylococcus aureus* in rice cake (Wang et al., 2017). This modeling for 1, 2, 3 and 4 log increase of *S. aureus* have been applied successfully. Therefore, probability of *S. aureus* growth in rice cake for certain log increases could be estimated. This procedure can also be applied for any lethal treatment (heat, pressure, disinfection) for any microorganisms in any food. The objective of this study was to demonstrate the usability of probabilistic modeling for HHP inactivation of microorganism for target log reductions.

2. The method

2.1. Data sets

Two published articles were used as the database: Erkmen (2009) inactivated *Salmonella* Typhimurium in broth at different pressure (200, 250, 300 and 350 MPa) and temperature (15, 25, 35 and 45 °C) levels for 0–50 min and Van Opstal et al. (2005) applied HHP (150–600 MPa; 5–45 °C; 0–60 min) to inactivate *Escherichia coli* in buffer and carrot juice. Data given in the figures of these studies were digitized using WinDIG 2.5 (Lovy, 2002) and were organized in Microsoft® Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheets. Complete list of parameters used for modeling are given in Table 1.

2.2. Modeling

Probabilistic model in the below forms were used:

$$logit\,(p) = c_0 + c_1 \bullet Press + c_2 \bullet Temp + c_3 \bullet t + c_4 \bullet Press \bullet Temp + c_5 \bullet Press \bullet t + c_6 \bullet Temp \bullet t + c_7 \bullet Press \bullet Temp \bullet t \quad (1)$$

or

$$logit\,(p) = \alpha_0 + \alpha_1 \bullet Press + \alpha_2 \bullet Temp + \alpha_3 \bullet lnt + \alpha_4 \bullet Press \bullet Temp + \alpha_5 \bullet Press \bullet lnt + \alpha_6 \bullet Temp \bullet lnt + \alpha_7 \bullet Press \bullet Temp \bullet lnt \quad (2)$$

where, p is the probability of a selected target log-reduction, $logit\,(p) = \ln[p/(1-p)]$, *Press* is pressure (MPa), *Temp* is temperature (°C), t is time (min) and c_0-c_7, α_0- α_7 are the coefficients to be estimated. The logarithm of time was used in the Eq.(2) because nonlinear relationship was often reported between microbial inactivation holding time (Koseki and Yamamoto, 2007; Koseki et al., 2009; Tamber, 2018). Modeling could be a useful tool to understand the effects of process parameters and their interactions (Khanipour et al., 2016) on microbial inactivation by HHP. Therefore, interaction terms were also included in the model equations.

Two target log reductions were selected for *S.* Typhimurium in broth which were 4 and 5 log-reductions, respectively. Only 5 log-reduction was used as a target for *E. coli* in buffer and carrot juice. For example, if 5 log reduction was obtained (at a certain pressure, temperature and time value for *E. coli* in buffer or carrot juice) then $p = 1$ and if not $p = 0$. Minimum 5 log reductions should be attained for the pathogenic microorganisms (*E. coli* O157:H7) in juices (US FDA, 2004) that is why 5 log reduction was used as a target.

Modeling has been applied as follows: (i) main effects (pressure, temperature and time) were forced to stay in the model, (ii) if the interaction terms were insignificant ($P \geq 0.05$), they were removed from the model and regression was repeated without those terms. No insignificant term stayed in the model by the application of step (i) i.e., remaining coefficients in the model were all significant ($P < 0.05$) – see results section. The fitted achieving/not achieving the target log reduction interfaces for $p = 0.1$, 0.5 and 0.9 were plotted in Microsoft® Excel Solver.

2.3. Evaluation of model performance

Both SPSS (Version 22, Chicago, IL, USA) and SigmaPlot (Version 12, Chicago, IL, USA) were used for model fitting because these software programs give different indices of goodness-of-fit. The goodness-of-fit of the models were assessed by (i) $-2{\cdot}\ln L$ with L the likehood in its optimum, (ii) Pearson Chi-square statistic, (iii) Likelihood ratio test statistics, (iv) Hosmer-Lemeshow statistic. The goodness-of-fit statistics indicate if the fitted model is correct or not (Hosmer et al., 2013). First statistic ($-2{\cdot}\ln L$) can be used to rank the models according to their goodness-of-fit, but does not give an idea about the adequacy of the model fit (Dang et al., 2010). Small values of $-2{\cdot}\ln L$ correspond to better fitting models (Gysemans et al., 2007). If small values and corresponding large values of P are obtained for Pearson Chi-square statistic, this indicates a good agreement between the logistic regression equation and the data. On the other hand, small P values for likelihood ratio test statistic indicate a good fit between the logistic regression equation and the data. The Hosmer-Lemeshow P value indicates how well the logistic regression equation fits the data by comparing the number of individuals with each outcome with the number expected based on the logistic equation. If Hosmer-Lemeshow statistic takes a small value or its corresponding P value is high, then the model fits the data adequately.

Models were also evaluated by (i) maximum rescaled R^2 (Nagelkerke, 1991) statistic and (ii) percent concordant (Gysemans et al., 2007). The R^2 indicates how useful the independent variables are in describing the response variable (Bewick et al., 2005) and percent concordance reflects the correspondence between observed and fitted values (Dang et al., 2010).

2.4. Model validation

The model [whether Eq. (1) or Eq. (2)] which has the better goodness-of-fit indices were used for validation. Predicted probabilities obtained from the models were compared with the data available in literature. Data given in either tables or figures were used. Data in figures were digitized and used as described above.

3. Results and discussion

3.1. Model development

Following logistic regression models [Eqs. (3) and (4)] were developed for *S.* Typhimurium in broth for 4 and 5 log reductions, respectively:

$$ln\left(\frac{p}{1-p}\right) = -50.71 + 0.103 \bullet Press + 0.165 \bullet Temp + 5.31 \bullet lnt \quad (3)$$

$$ln\left(\frac{p}{1-p}\right) = 32.44 - 0.203 \bullet Press + 0.284 \bullet Temp - 24.68 \bullet lnt + 0.104 \bullet P \bullet lnt \quad (4)$$

and following logistic regression models [Eqs. (5) and (6)] were developed for *E. coli* in buffer and carrot juice, respectively for 5 log reductions:

Table 1
Data used for logistic regression.

Pressure (MPa)	Temperature (°C)	Time (min)	Medium	Reference
200, 250	15, 25, 35, 45	5, 10, 15, 20, 25, 30, 40, 50	Tryptone soy broth	Erkmen (2009)
300	15, 25, 35	5, 10, 15, 20, 25, 30, 40, 50		
300	45	5, 10, 15, 20, 25		
350	15	5, 10, 15, 20, 25, 30, 40, 50		
350	25	5, 10, 15, 20, 25, 30, 40		
350	35	5, 10, 15, 20, 25, 30		
350	45	5, 10, 15, 20		
200, 250, 300,	5, 20	1, 2, 4, 8, 15, 35, 60	Hepes-buffer	Van Opstal et al. (2005)
350, 400, 450	5, 20	1, 2, 4, 8, 15, 35, 60		
500	5	1, 2, 4, 8, 15, 35		
200, 225, 250,	10	1, 2, 4, 8, 15, 35, 60		
300,350	10	1, 2, 4, 8, 15, 35, 60		
400,450	10	1, 2, 4, 8, 15, 35		
500	20	1, 2, 4, 8, 15		
250, 300, 350	30	1, 2, 4, 8, 15, 35, 60		
400	30	1, 2, 4, 8, 15, 35, 60		
450	30	1, 2, 4, 8		
200, 250, 300	40	1, 2, 4, 8, 15, 35, 60		
350	40	1, 2, 4, 8		
150, 175, 200	45	1, 2, 4, 8, 15, 35, 60		
250	45	1, 2, 4, 8, 15		
400, 500, 550	5, 10	1, 2, 4, 8, 15, 35, 60	Carrot juice	Van Opstal et al. (2005)
600	5, 10	1, 2, 4, 8, 15, 35		
300, 350, 400,	20	1, 2, 4, 8, 15, 35, 60		
450, 500	20	1, 2, 4, 8, 15, 35, 60		
550	20	1, 2, 4, 8, 15, 35		
600	20	1, 2, 4, 8		
300, 350, 400	30	1, 2, 4, 8, 15, 35, 60		
450	30	1, 2, 4, 8, 15, 35, 60		
500	30	1, 2, 4, 8, 15		
250, 300	40	1, 2, 4, 8, 15, 35, 60		
350	40	1, 2, 4, 8, 15		
400	40	1, 2, 4, 8		
200, 250	45	1, 2, 4, 8, 15, 35, 60		
300	45	1, 2, 4, 8, 15		

$$ln\left(\frac{p}{1-p}\right) = -83.45 + 0.128 \bullet Press + 1.46 \bullet Temp + 15.24 \bullet lnt - 0.0014 \bullet Press \bullet Temp - 0.015 \bullet Press \bullet lnt - 0.251 \bullet Temp \bullet lnt \quad (5)$$

$$ln\left(\frac{p}{1-p}\right) = -137.1 + 0.196 \bullet Press + 2.52 \bullet Temp - 1.48 \bullet t - 0.0028 \bullet Press \bullet Temp + 0.0034 \bullet Press \bullet t + 0.02 \bullet Temp \bullet t \quad (6)$$

The coefficients of the models with their standard error and P values are given in Table 2. Note that regression was applied with all coefficients existing in a model and then it was applied step by step by removing one insignificant term at a time until the coefficients in the model were all significant ($P < 0.05$). The goodness-of-fit indices of the models applied [Eqs.(3), (4), (5) and (6)] are listed in Table 3. It can be seen that models produced good fits for the data being handled.

The use of logarithm of time instead of time yielded better models (results not shown) for *S.* Typhimurium in broth and *E. coli* in buffer since the relationship between microbial inactivation and holding time was nonlinear (Erkmen, 2009; Van Opstal et al., 2005). On the other hand, logarithmic transformation could not be used for *E. coli* in carrot juice. This is not surprising because a linear relationship was observed between microbial inactivation and holding time for *E. coli* in carrot juice (Van Opstal et al., 2005).

Percent concordant, fail-dangerous and fail-safe ratios were also given in Table 3. The model is unsuccessful whether it is fail-safe or dangerous (Ratkowsky, 2004). In case of growth/no growth modeling, fail-safe represents a model that tells you that a microorganism in a food product grow under the conditions which should not be grown. This means that according to the extreme fail-safe model, consumption of the mentioned food product is unsafe (therefore it should be avoided or destroyed) which is in fact safe to eat (Ratkowsky, 2004). However, in case of modeling the target log reduction, the situation is completely different: fail-safe refers to actually achieving the target log reduction, 5 log for example, at a specific pressure, temperature and time, but the model gives the wrong outcome i.e., 5 log reduction cannot be achieved. In such a case a new treatment with a higher pressure, temperature and time combination would be applied to inactivate the "already inactivated" microorganism which is, of course, waste of time and energy, but this does not mean waste of the food as in the case of fail-safe in growth/no growth modeling. It is best to reach % 100 concordant as much as possible, but this cannot be put into practice. Therefore, fail-safe percentage can be also important together with percent concordant in the modeling of target log reduction.

Probability of time required to reach 4 and 5 log reductions of *S.* Typhimurium in broth at 320 MPa, 30 °C predicted by Eqs. (3) and (4) is shown in Fig. 1. It was clear that at a constant time higher probabilities were obtained for 4 $\log_{10}$ reduction than 5 $\log_{10}$ reduction. Probability distributions predicted by Eqs. (5) and (6) for 5 $\log_{10}$ reductions of *E. coli* in buffer and carrot juice at 25 °C for 5 min is shown in Fig. 2. Difference between buffer and carrot juice could be visualized: higher pressure values were needed to obtain high probabilities in carrot juice than in buffer. These pressure, temperature and time levels were selected within the interpolation region and predicted probabilities with these values were plotted against time for *S.* Typhimurium in broth (4 and 5 log reductions) and pressure for *E. coli* in buffer and carrot juice (5 log reduction).

Both in Figs. 1 and 2, $p = 0.1$, $p = 0.5$ and $p = 0.9$ were marked. The region of $p > 0.5$ can be assigned as "likely to achieve the target log reduction". Fig. 3 shows the effects of pressure and holding time at 45 °C on achieving 4 $\log_{10}$ reduction of *S.* Typhimurium in broth and Fig. 4 shows the effects of pressure and holding time at 30 °C on achieving 5 $\log_{10}$ reduction of *E. coli* in carrot juice. It could be said that

Table 2
Estimated coefficients of the logistic regression for selected log-reductions.

Microorganism	Medium	Target log-reduction	Coefficients	Estimates	Standard error	*P* value	Reference
Salmonella Typhimurium	Tryptone soy broth	4	c_0	−50.71	11.79	< 0.001	Erkmen (2009)
			c_1	0.103	0.024	< 0.001	
			c_2	0.165	0.052	0.002	
			c_3	5.31	1.34	< 0.001	
			c_4	N.S.[a]	–	–	
			c_5	N.S.	–	–	
			c_6	N.S.	–	–	
			c_7	N.S.	–	–	
Salmonella Typhimurium	Tryptone soy broth	5	c_0	32.44	12.75	0.047	Erkmen (2009)
			c_1	−0.203	0.081	0.012	
			c_2	0.284	0.095	0.003	
			c_3	−24.68	8.823	0.005	
			c_4	N.S.	–	–	
			c_5	0.104	0.035	0.003	
			c_6	N.S.	–	–	
			c_7	N.S.	–	–	
Escherichia coli	Hepes-buffer	5	c_0	−83.45	20.164	< 0.001	Van Opstal et al. (2005)
			c_1	0.128	0.0326	< 0.001	
			c_2	1.46	0.372	< 0.001	
			c_3	15.24	4.014	< 0.001	
			c_4	−0.0014	0.000451	0.003	
			c_5	−0.015	0.00513	0.004	
			c_6	−0.25	0.0639	< 0.001	
			c_7	N.S.	–	–	
Escherichia coli	Carrot juice	5	α_0	−137.11	51.258	0.007	Van Opstal et al. (2005)
			α_1	0.196	0.0742	0.008	
			α_2	2.52	0.955	0.008	
			α_3	−1.48	0.601	0.014	
			α_4	−0.0028	0.00112	0.012	
			α_5	0.0034	0.00115	0.003	
			α_6	0.0202	0.00818	0.014	
			α_7	N.S.	–	–	

[a] Not significant ($P > 0.05$).

interfaces i.e., probability of achieving or not achieving target log reductions were coherent with the experimental data.

This study showed that probabilistic modeling can be used for HHP inactivation of any microorganism (in any food) for which target log-reduction is pre-specified. Note that, the data taken from literature were generated for kinetic modeling not for the probabilistic modeling. Therefore, longer treatment times (> 10 min) were also included in the modeling study. Moreover, only three processing parameters were used; however, intrinsic factors of the food such as pH, water activity as well as initial number of microorganisms can also be used as the other parameters for the modeling purposes. Koseki et al. (2009) applied logistic regression to determine survival/death interface of *Cronobacter* spp. during HHP processing. Their parameters were pressure, temperature, holding time, inoculum level of *Cronobacter* spp. and medium (broth or infant formula). What is interesting about the study of Koseki et al. (2009) is that they also used the resulting probability model to determine required log reduction similar to this study. However, they designed their model according to survival ($p = 0$) or death ($p = 1$) and then the best model obtained was used to find probability of inactivation for achieving a required log reduction. In our case, modeling was directly designed as achieving the target (4 or 5 log) log reduction ($p = 1$) or not ($p = 0$).

3.2. Model validation

3.2.1. S. Typhimurium validation in broth

Alpas et al. (2000) used HHP (207, 276, 345 MPa; 25, 35, 45, 50 °C; 5 and 10 min) to inactivate *S.* Tyhimurium in peptone water. These data (except 50 °C to avoid extrapolation) were used for the validation study of *S.* Tyhimurium for 4 and 5 log reductions [Eqs. (3) and (4)] in broth. The very same temperature and time data of Alpas et al. (2000) were used for the development of the models in this study, but pressure levels were unique to Alpas et al. (2000). The model for 4 log reductions [Eq. (3)] predicted 12 out of 18 data points correctly. Incorrect predictions were for 207 MPa, 45 °C for 5 and 10 min, and 276 MPa, 35 and 45 °C for 5 and 10 min. In these cases, > 4 log reductions were observed; however, probabilities obtained by the model [Eq. (3)] were < 0.2 indicating fail-safe predictions.

For 5 log reduction [Eq. (4)] of *S.* Tyhimurium, 13 out of 18 points were correctly predicted. The data points that were incorrectly

Table 3
Goodness-of-fit indices of the fitted models.

	Eq.(3)	Eq.(4)	Eq.(5)	Eq.(6)
$-2 \ln L$	39.185	29.503	73.806	24.377
Pearson chi-square statistics	42.894 ($P = 1.000$)	44.606 (P = 1.000)	111.000 (P = 1.000)	37.825 (P = 1.000)
Likelihood ratio test statistics	117.690 ($P \leq 0.001$)	104.295 (P ≤ 0.001)	117.279 (P ≤ 0.001)	122.378 (P ≤ 0.001)
Hosmer-Lemeshow statistics	2.938 ($P = 0.938$)	1.121 ($P = 0.997$)	0.921 ($P = 0.999$)	0.504 (P = 1.000)
Maximum rescaled R^2	0.858	0.865	0.710	0.886
Percent concordant	% 91.6	% 94.1	% 92.0	% 97.1
Fail-safe	% 4.2	% 3.4	% 5.3	% 1.2
Fail-dangerous	% 4.2	% 2.5	% 2.7	% 1.7

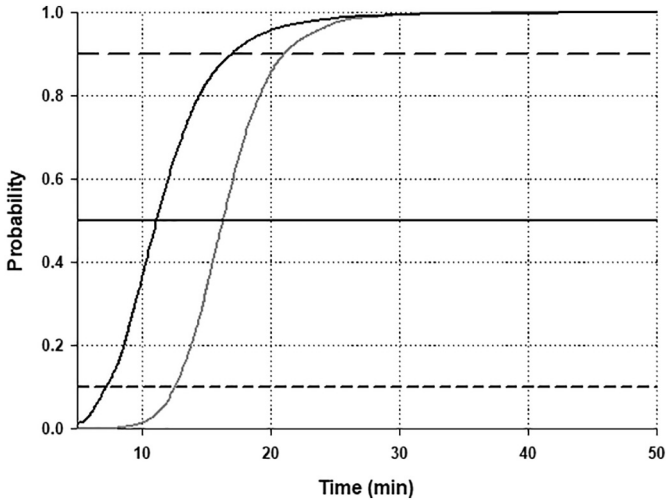

Fig. 1. Probability of time required for 4 log reduction [black solid curve – generated by using Eq. (3)] and 5 log reduction [gray solid curve– generated by using Eq. (4)] of *Salmonella* Typhimurium in broth at 320 MPa, 30 °C. Short dashed line at the bottom, solid line at the center and long dashed line at the top represent the probability values of 0.1, 0.5 and 0.9, respectively.

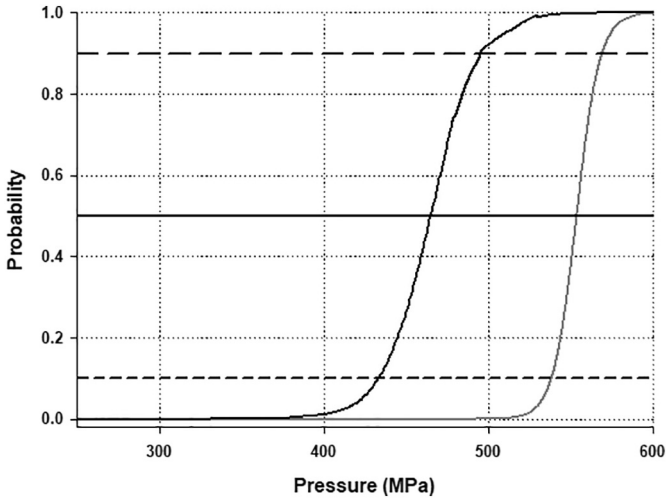

Fig. 2. Probability of pressure required for 5 log reduction of *Escherichia coli* in buffer [black solid curve – generated by using Eq. (5)] and in carrot juice [gray solid curve– generated by using Eq. (6)] at 25 °C, 5 min. Short dashed line at the bottom, solid line at the center and long dashed line at the top represent the probability values of 0.1, 0.5 and 0.9, respectively.

predicted were 276 MPa, 45 °C for 5 and 10 min, 345 MPa, 25 °C for 5 and 10 min, and 345 MPa, 35 °C for 5 min. Once again, model [Eq. (4)] predictions were on the fail-safe side: probabilities were all < 0.5, but > 5 log reductions were obtained.

It is known that there may be variation in resistance to HHP even among the different strains of bacteria (Alpas et al., 1999; Benito et al., 1999). The models [Eqs. (3) and (4)] developed for *S.* Tyhimurium KUEN 1357 in broth, but predictions were done for *S.* Tyhimurium E21274 VL in peptone water. The reasons of incorrect predictions may be the strain variation and use of different media.

3.2.2. *E. coli* validation in carrot juice

Pilavtepe-Çelik et al. (2009) applied HHP to inactivate *E. coli* O157:H7 in carrot juice (200–350 MPa, 40 °C, 0–40 min). From the study of Pilavtepe-Çelik et al. (2009) 350 MPa, 40 °C, 2.5, 7.5 and 12.5 min were used to validate the model [Eq. (6)] obtained. Note that pressure and temperature (also some of the time values) values of

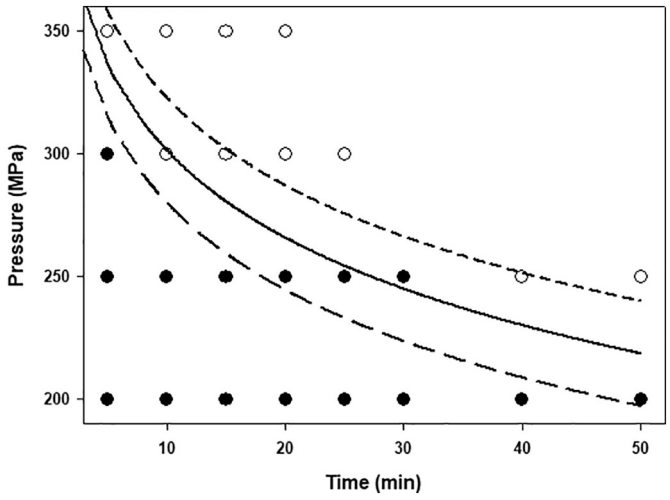

Fig. 3. Achieving/not achieving 4 log reduction interface of *Salmonella* Typhimurium in broth at 45 °C. Black and white circles represent not achieving and achieving 4 log reduction. Lines represent the model predictions $p = 0.1$ (short dashed), $p = 0.5$ (solid), $p = 0.9$ (long dashed).

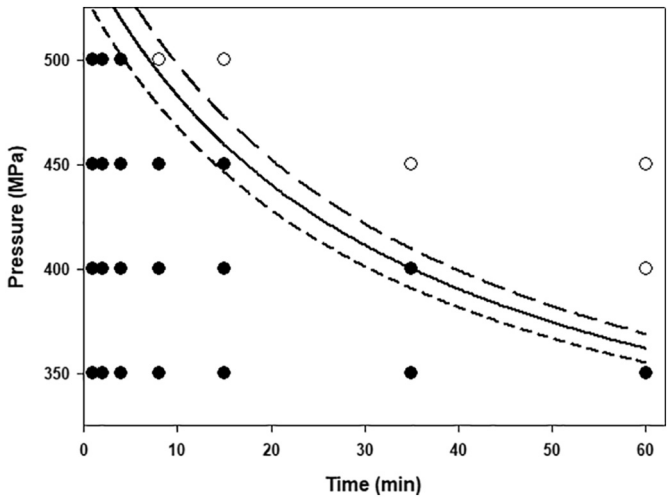

Fig. 4. Achieving/not achieving 5 log reduction interface of *Escherichia coli* in carrot juice at 30 °C. Black and white circles represent not achieving and achieving 5 log reduction. Curves represent the model predictions $p = 0.1$ (short dashed), $p = 0.5$ (solid), $p = 0.9$ (long dashed).

Pilavtepe-Çelik et al. (2009) coincided with the ones used in this study therefore, time values which were not coincided were obtained and used for the validation study.

The probabilities obtained for 2.5, 7.5 and 12.5 min for carrot juice were about 0.003, 0.035 and 0.32, respectively indicating that 5 $\log_{10}$ inactivation of *E. coli* O157:H7 in carrot juice is not likely to achieve ($p \leq 0.5$) at these pressure (200–350 MPa) and temperature (40 °C) levels. In fact, inactivations were even less 4 $\log_{10}$. Van Opstal et al. (2005) used a pressure sensitive strain of *E. coli*, but studies have shown that pathogenic strains of *E. coli* (O104:H4 and O157:H7) have higher pressure resistance than the non-pathogenic strain (DSM1116) in buffer and carrot juice (Reineke et al., 2015). Although different bacteria (*E. coli* K-12 strain MG1655 and *E. coli* O157:H7 933) were used for model development and validation, this did not affect the predictions by the model developed [Eq. (6)]. Nevertheless, prediction studies should not be done with only one strain in one or two food since strains of the same species exhibit substantial variability in pressure resistance (Alpas et al., 1999; Benito et al., 1999; Liu et al., 2015; Tamber, 2018). The resistance of microorganisms to pressure is not only variable between

strains but also dependent on the food matrix (Gänzle and Liu et al., 2015). Therefore, the validation done in this study may not be reliable since models were developed for different strains (*S.* Tyhimurium KUEN 1357 and *S.* Tyhimurium E21274 VL, and *E. coli* MG1655 and *E. coli* O157:H7) in different buffer systems (broth and peptone water for *S.* Tyhimurium), but since the aim was to demonstrate the usability of probabilistic modeling for HHP inactivation of microorganism for target log reductions both model development and validation steps were displayed with the published data.

4. Concluding remarks

This study has indicated that the probabilistic modeling for target log reductions can be useful tool for HHP inactivation of microorganisms. Models were developed and tried to be validated by using the published data; however, design of new experiments according to this modeling technique could be possible and beneficial to food industry. Target log reductions for relevant cocktail of pressure-resistant strains of the target species, holding time, temperature and pressure levels can be selected at various levels. Moreover, pH, water activity, concentrations of different chemicals (lactic acid, nisin, etc.) can also be used as the model parameters.

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