Determination of the survival kinetics of Salmonella spp. on the surface of ripened raw milk cheese during storage at different temperatures

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Summary The aim of this study was to determine the survival kinetics of Salmonella enterica subsp. enterica in ripened raw milk cheese. Cheese samples inoculated with S. enterica subsp. enterica were stored at 5, 15 and 25 °C and analysed in terms of physico-chemical and microbiological characteristics. Three primary models were used to estimate the kinetic parameters of S. enterica subsp. enterica. The secondary Arrhenius model was used to establish the relationship between temperature and parameter a of the Weibull model. Additionally, prediction of S. enterica subsp. enterica survival as a function of storage temperature was made. S. enterica subsp. enterica growth was inhibited during storage, and bacteria survived for an extensive period of time at high number (60 day at 5 °C, 26 day at 25 °C). The storage temperature significantly influenced the inactivation rate of Salmonella in raw milk ripened cheese and proceeded faster at 25 °C compared to remaining storage temperature. Obtained results suggest that contamination by Salmonella in raw milk cheese might result in residual risk.

Keywords Food safety, mathematical modelling, raw milk cheese, Salmonella enterica subsp. enterica.

Introduction Consumers more often are interested in unprocessed or minimally processed food products. Raw milk and raw milk products belong to the minimally processed food category. Raw milk cheeses are the most frequently consumed type of dairy products among products made from unpasteurised milk and constitute an oldest known type of manufactured food products (Little et al., 2008; Verres et al., 2008).

Pasteurisation process increases both safety and quality of dairy products. The process also causes adverse effects such as protease and lipase inactivation and kills the naturally present microorganisms (Enterococcus spp., Lactococcus spp., Leuconostoc spp., Lactobacillus spp.) that are responsible for the specific taste of cheeses made from raw milk (Grappin & Beuvier, 1997; Pacheco & Galindo, 2010; Masoud et al., 2012; Yoon et al., 2016). As a result of the presence of native microbiota, raw milk cheeses exhibit higher amounts of volatile compounds such as carboxylic acids, alcohols and esters compared to pasteurised milk cheeses (Ocak, Javidipour, & Tuncturk, 2015; Velente et al., 2018). Some consumers consider raw milk cheeses as ‘risky’ products as a number of food-borne disease outbreaks associated with raw milk cheeses consumption have been reported worldwide (Licitra, Caccamo, & Lortal, 2019; Verraes et al., 2014).

Salmonella spp. is considered, among others, as the main microbiological hazard associated with raw milk consumption (Pyz-Lukasik et al., 2015; Asselt et al., 2017). The majority of the outbreaks due to the consumption of raw milk cheeses was caused by Salmonella, followed by Verotoxigenic Escherichia coli strains (VTEC). The pathogen can contaminate raw milk, and as there is no pasteurisation step before cheese production, it will not be inactivated (Almeida et al., 2007; Verraes et al., 2015).

A dose–response curve estimates the probability of developing a disease on the basis of relationship between the number of cells and the chance of disease occurrence (Verraes et al., 2015). EFSA BIOHAZ Panel in the framework of a risk ranking exercise of foods of non-animal origin (FoNAO) allocated the highest score 3 to Salmonella which stands for a dose–
response curve where the pathogen can cause illness in low number (EFSA Panel on Biological Hazards (BIOHAZ) 2013; Da Silva et al., 2015). *Salmonella* is also recognised by the European Legislation (EC, 2007) as food safety criteria in the cheeses made from raw milk or milk that has undergone a lower heat treatment than pasteurisation where the absence of the microorganism in 25 g is required.

The growth of bacteria depends on external factors (environmental parameters) and the internal characteristics of the food products (pH, aw, etc.). The most important environmental parameter that governs the microbial growth is temperature (Juneja, Melendres, Huang, Subbah, & Thipperaddi, 2009). Predictive microbiology is a multidisciplinary science that deals with predicting the response of microorganisms, especially pathogenic ones, to environmental conditions. Survival curves of microbial population have been proven to be sigmoidal or semisigmoidal with a ‘shoulder’ and/or tailing region. Accordingly, growth models, such as Baranyi and Gompertz models, have been shown to be successfully applicable to describe bacterial inactivation. Nonlinear survival models, such as Weibull or log-linear, have been satisfactorily applied to model the behaviour of *Salmonella* spp. in different food matrices (Farakos et al., 2016; Pasquali, Klein, Reich, Manfreda, & Valero, 2016). In the last years, predictive models were generated for many dairy products. Most recently, predictive microbiology was applied to determine the thermal inactivation kinetics of *Paenibacillus sanguinis* and *Clostridium sporogenes* in full and low fat ‘requeijao cremoso’ (Oliveira et al., 2018). Nowadays, predictive microbiology is also applied to investigate interaction between microorganisms. Valik et al. (2018) studied the simultaneous growth of a starter culture of lactic acid bacteria and *Staphylococcus aureus* in milk.

The aim of this study was to investigate the ability of *S. enterica* subsp. *enterica* to grow or survive in raw milk ripened cheese throughout storage at 5, 15 and 25 °C. The observed data were fitted to predictive nonlinear survival models in order to calculate the kinetic parameters. Finally, the influence of temperature on the behaviour of pathogen during different storage temperature was established. Simultaneously, the microbiological and physico-chemical analyses of the studied cheese were performed during storage.

**Materials and methods**

**Raw milk ripened cheese**

Cheese wheels (ca. 2.5 kg each) produced from raw milk used for the study were acquired directly from the local manufacturer. It was a hard cheese with a ripening period of ca. 3–6 months. Cheese was produced from a non-standardised whole milk (milk with a fat content that has not been altered since the milking stage) with addition of the following ingredients: starter cultures, rennet, calcium chloride (stabiliser) and salt. Cheese was analysed for the *Salmonella* spp. absence according to ISO 6579:2002 (ISO, 2002).

Cheese was aseptically crumbled and portioned into 25 g samples and placed in the sterile polyethylene VWR blender bags with non-woven polyester filter (VWR International Sp. z o.o., Gdansk, Poland).

**Salmonella** spp. strains, growth conditions and inoculum preparation

A combination of five strains of *Salmonella* spp. isolated from dairy products (*Salmonella* Typhimurium, *Salmonella enterica* subsp. *enterica*) and one reference strain (*Salmonella enterica* subsp. *enterica* (D) ATCC 13076, MicroBioLogics, St. Cloud, Minnesota, USA) were used to prepare a cocktail of *Salmonella* spp. strains used for artificial contamination of cheese samples. *Salmonella* spp. strains originated from food products were isolated and confirmed at the Chair of Industrial and Food Microbiology (Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland).

Strains were stored individually at −80 °C in BHI broth (Merck, Warsaw, Poland) with 20% (v/v) glycerol (Merck, Warsaw, Poland) and grown by transferring 0.1 mL of the culture to 10 mL of BHI broth followed by 24 h incubation at 37 °C (ICP500, Memmert GmbH+Co. KG, Schwabach, Germany). The second passage was performed by transferring 0.1 mL from each 24 h culture to 10 mL BHI broth followed by incubation (37 °C for 24 h). The initial concentration of microorganisms in each working subculture was determined by traditional plate counts method using the BHI agar (Merck, Warsaw, Poland). The cocktail of *Salmonella* spp. strains was prepared by combining equal volumes (3 mL) of each activated strain. Estimated final concentration of *Salmonella* spp. in the cocktail was ~9 log cfu mL⁻¹.

**Cheese inoculation and storage**

The cocktail of *Salmonella* spp. strains was serially diluted to give a final concentration of ~6.5 log cfu g⁻¹ of cheese. Samples of cheese (25 g) placed in a sterile bag were inoculated with 100 µL of prepared mixture of microorganisms, packaged and stored at three temperatures: 5, 15 and 25 °C (ICP500, Memmert GmbH+Co. KG, Schwabach, Germany). Inoculated cheese samples were stored for 26, 42 and 60 days, respectively, at 25, 15 and 5 °C. Sampling time depended on the applied storage temperature:
5 °C – every three days, 15 °C – every two days and 25 °C – each day.

Microbial enumeration

At appropriate time intervals, depending on the applied storage temperature, the number of Salmonella spp. was checked by analysing three independent cheese samples using a selective medium XLD agar (Merck, Warsaw, Poland). At each sampling day, the bags containing inoculated cheese were aseptically opened, followed by addition of 225 mL of peptone water (Merck, Warsaw, Poland). The samples were homogenised in the lab blender BagMixer® 400W (Intersience, St Nom la Bretêche, France). From each sample, the serial dilutions were prepared using 9-mL dilution blanks of peptone water. The enumeration of Salmonella was performed by spread plating on XLD agar in triplicate using 0.1 mL of the appropriate dilutions and distributed evenly over the surface of medium. The plates were aerobically incubated at 37 °C for 48 h and enumerated. The mean from three platings of each sampling point (cfu g⁻¹) was used to establish the growth kinetics. The experiments were conducted in three replicates for each incubation temperature, using independent batches of cheese.

Simultaneously, at each sampling day the number of (i) coliform bacteria and E. coli (Chromocult® Coliform Agar), (ii) total aerobic microbial count (TSA with polysorbate 80 and lecithin), (iii) S. thermophilus (M 17 agar acc. to TERZAGHI), (iv) Lactobacillus (MRS agar acc. ISO 15214 + Anaerocult C) and (v) Enterobacteriaceae (VRBD agar) was checked. All media were acquired from Merck (Warsaw, Poland) and used according to the manufacturer’s instruction.

The number of yeasts and moulds was determined using the TEMPO® YM selective tests compatible with TEMPO® system (bioMérieux). The inoculated cheese samples were decimally diluted in the peptone water (Merck, Warsaw, Poland). The TEMPO® YM test requires to hydrate the selective media by adding 3 mL of sterile water. After adding a 1 mL of appropriately diluted product, a scan was made. Prepared samples were placed in the TEMPO® Filler component. After reading the data and closing the cards, the stands containing filled cards were transferred to the incubators and stored at 25 °C for 72 h. From the cards, filler station data were sent to the reading station. After incubation period, the cards were placed in TEMPO® Reader station, where the data were saved. The last stage of work was validation of the obtained results.

Physico-chemical measurements

The chemical composition (protein, fat, dry matter, salt, water) of the raw milk ripened cheese was measured using a FoodScan (Foss, Warsaw, Poland). Water activity was measured with Hygropalm HP23-AW (Rotronic AG, Bassersdorf, Germany). The chemical composition and water activity of cheese were measured at the beginning and at the end of the storage period. The pH of the cheese was measured with a Lab 860 electrode (Schott® Instruments Inc., Mainz Germany). The acidity of cheese was measured during whole storage period at each temperature (5 °C – every three days, 15 °C – every two days, 25 °C – each day).

Kinetics of Salmonella spp. survival

Primary modelling

At each temperature, 21–22 bacterial-count points transformed to log₁₀ values were plotted against time (in days) in MS Excel v2010 (Microsoft). To elaborate the primary survival/inactivation models, the following mathematical functions were used:

- The Baranyi model (Baranyi, Roberts, & McClure, 1993; Baranyi & Roberts, 1994):

\[ N(t) = N_0 + \mu_{\text{max}} F(t) - \ln \left( 1 + \frac{e^{\mu_{\text{max}} F(t) - 1}}{e^{N_{\text{max}} - N_0}} \right) \]  

\[ F(t) = t + (1 / v) \cdot \ln \left( e^{-vt} + e^{-h_0} - e^{(-vt-h_0)} \right) \]

where \( N(t) \) is the cell concentration at time \( t \) (In cfu g⁻¹), \( N_0 \) is the initial cell concentration (In cfu g⁻¹), \( N_{\text{max}} \) is the maximum cell concentration (In cfu g⁻¹), \( \mu_{\text{max}} \) is the maximum specific growth rate (h⁻¹), \( v \) is the rate of increase of the limiting substrate, and \( h_0 \) is a product of \( \mu_{\text{max}} \cdot \lambda \), where \( \lambda \) denotes the duration of the lag phase in days.

The function \( F(T) \) plays the role of a gradual delay in time. The Baranyi model has four parameters: \( N_0 \), \( h_0 \), \( N_{\text{max}} \) and \( \mu_{\text{max}} \). Model assumes that the course of the growth, \( N(t) \), is affected by the initial microbial cell concentration, \( N_0 \), as well as the physiological state of the inoculum (Baranyi, Roberts, & McClure, 1993; Baranyi & Roberts, 1994).

Baranyi et al. (1996) propose to consider an inactivation curve as the mirror image of a growth curve. The model in case of survival/inactivation curves is based on four parameters: shoulder period, the inactivation rate (log cfu g⁻¹ d⁻¹), initial bacterial number (upper asymptote), and final bacterial number (lower asymptote; Skandamis & Nychas, 2000). The Baranyi model was fitted to the observed survival data of Salmonella spp. by using the DMFit MS Excel add-in (Food Safety Centre, Hobart, Australia).

- The Weibull model (Mafart, Couvert, Gaillard, & Leguerinel, 2002):

\[ \log_{10} N(t) = \log_{10} N_0 - (t / \delta)^p \]

where \( N_0 \) represents initial population, parameter \( p \) characterises the shape of the curves, and \( \delta \) represents the продолжительность периода. В модели инактивации используются четыре параметра: плечевой период, инактивационная скорость (логарифм колонных единиц г⁻¹ д⁻¹), начальная бактериальная численность (верхняя асимптота), и конечная бактериальная численность (нижняя асимптота; Skandamis & Nychas, 2000). Модель Барани и др. была применена для оценки выживаемости данных Salmonella spp. с помощью программы DMFit MS Excel add-in (Food Safety Centre, Hobart, Australia).

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corresponds to the times for the first decimal reduction in microbial population (10-fold reduction of the surviving population).

Parameter δ can be called time of first decimal reduction. This is distinguished from the D-value, which is derived from the first-order kinetic and represents the time of decimal reduction, regardless of the time of heating. The significance of the δ value is restricted to the first decimal reduction of surviving cells from N₀ to N₀/10 (Mafart, Couvert, Gaillard, & Leguerinel, 2002).

The Weibull model has been previously used to describe bacterial nonlinearity survival curves of Salmonella enterica subsp. enterica in inactivation by thermal and non-thermal processes (de Oliveira, Soares, & Piccoli, 2013).

- The log-linear + tail model (Geeraerd, Herremans, & Van Impe, 2000):

\[ N = (N_0 - N_{res}) \cdot e^{-k_{max}t} + N_{res} \]  

where N is the cell concentration at time t (cfu g⁻¹), \( N_0 \) represents initial population (cfu g⁻¹), \( N_{res} \) is the residual population density (cfu g⁻¹), and \( k_{max} \) is the maximum inactivation rate (d⁻¹).

The log-linear part of the inactivation curve is modelled with first-order kinetics (\( k_{max} \)). The tailing effect is reflected in the additional factor (\( N_0 - N_{res} \)), which implies the existence of a subpopulation \( N_{res} \) (Geeraerd, Herremans, & Van Impe, 2000).

The freeware add-in GInaFiT v1.6 (Geeraerd, Valdramidis, & Van Impe, 2005) was used for the fitting procedure of the Weibull and log-linear models and statistical calculations.

**Secondary modelling**

The secondary Arrhenius model was used to establish the relationship between temperature and parameter \( \alpha \) of the Weibull model (Angelidis, Papageorgiou, Tyrovouzis, & Stoforos, 2013):

\[ \alpha(T) = \alpha_{ref} \cdot e^{-E_a/(Rg \cdot T_{ref})} \]  

where \( \alpha(T) \) is the \( \alpha \) value at temperature \( T \), \( \alpha_{ref} \) is the \( \alpha \) value at a constant reference temperature, \( T_{ref} \), \( E_a \) is the activation energy (J mol⁻¹), and \( R_g \) is the universal gas constant, 8.314 J (mol K)⁻¹. Both temperatures, namely \( T \) and \( T_{ref} \), represent absolute temperature expressed in K. A reference temperature of 283.15 K (i.e. 10 °C) was applied.

The reduction ratio of bacterial cells \( N(t)/N_0 \) can be considered as dependent variable. In order to establish the potential cell reduction at other storage temperature, the Eqs. (3) and (5) can be combined to the following equation (Angelidis et al., 2013):

\[ \log(N(t)/N_0) = - \left( \frac{t}{\alpha_{ref} \cdot e^{-E_a/(Rg \cdot T_{ref})}} \right)^\beta \]  

A nonlinear regression procedure was performed using an add-in Solver (MS Excel) which involves the nonlinear optimisation generalised reduced gradient (GRG2).

The last stage of work was to establish a relationship between temperature and parameter \( \beta \) (Valero et al., 2014):

\[ \beta = -(a \cdot T) + b \]  

**Statistical analyses**

The mean square error (MSE) and the correlation coefficient (\( R^2 \)) were automatically reported by the GInaFiT tool.

The goodness of fit of the fitted models was evaluated, including the calculation of Akaike’s information criterion (AICc), with bias adjustment for small sample size (Burnham & Anderson, 2002):

\[ \text{AIC}_c = -2 \cdot \ln(\text{likelihood}) + 2 \times K + [2 \times K \times (K + 1)] \cdot (n - K - 1)^{-1} \]  

where \( n \) is the number of observations and \( K \) is the number of the model’s parameters. The smaller the value of \( \text{AIC}_c \), the better the description of observed data by the mathematical model.

An analysis of variance (F-test two samples for variances) was used for analyses of the data (MS Excel). The obtained results were considered significant at \( P < 0.05 \).

The fitting performance of the models was checked using the F-test. The following equation was used to calculate the f value:

\[ f = \frac{\text{MSE}_{model}}{\text{MSE}_{data}} \]  

where \( \text{MSE}_{model} \) is the mean square error of the of the model and the \( \text{MSE}_{data} \) is the mean square error of the data. The \( \text{MSE}_{data} \) value indicates the variation between replicates for a given experimental condition and is calculated according to the following formula:

\[ \text{MSE}_{data} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k} (\text{average log}_{10} N_i - \text{log} N_{ij})^2}{n - m} \]  

where \( n \) is the number of data points, \( m \) the number of time points, \( k \) is the number of replicates at each time point, average \( N_i \) is the mean value of the population at time point \( i \) (log CFU g⁻¹), and \( N_{ij} \) is the
population at time point $i$ for specific replicate $j$ (log CFU g$^{-1}$; Valero et al., 2014).

The calculated value was compared to $F$ table value at 95% confidence level. If the calculated value was smaller than the $F$ table value, the $F$-test was accepted and the model fitting was statistically acceptable.

**Results and discussion**

**Physico-chemical characteristics of ripened raw milk cheese during storage**

The analysed formulation of the raw milk cheeses was as follows: proteins – 27%, dry matter – 74%, salt – 1.7%, fat – 44%, and water – 26%. The measured $a_w$ values remained constant during storage (0.955 ± 0.007) without statistically significant differences ($P > 0.05$), irrespective of applied storage temperature. Figure 1 shows the changes of the pH value of the raw milk cheese stored at 5, 15 and 25 °C. The measured increase of the pH during storage from the initial value 5.35 was 0.5 ± 0.05 at 5 °C and 0.7 ± 0.06 at 15 and 25 °C. Due to the pH value close to a neutral, high water content and low amount of salt, the raw milk ripened cheese constituted a suitable substrate for the growth of the examined pathogen.

**Microbial characteristics of ripened raw milk cheese during storage**

The yeasts and moulds population remained constant (5.0 ± 0.11 log cfu g$^{-1}$) in the raw milk cheese during storage at all tested temperatures. Figure 2a-e shows the changes in the microbial population of the experimentally contaminated raw milk cheese during storage at 5, 15 and 25 °C. A decrease in the number of Lactobacillus sp. by 0.6 and 1.0 log was observed, respectively, at 5 and 15 °C. However, an increase of 1.1 log of Lactobacillus sp. was noticed at 25 °C (Fig. 2a). The number of coliforms and E. coli reduced by 2.1 and 0.6 log, respectively, at 5, 15 and 25 °C (Fig. 2b). Figure 2c shows the changes in the total aerobic count during storage. A decrease by 2.0, 0.9 and 1.7 log was observed, respectively, at 5, 15 and 25 °C. A decrease in S. thermophilus population by 1.8 and 1.5 log, respectively, at 5 and 15 °C was observed (Fig. 2c). However, the level of S. thermophilus remained unchanged (6.6 log) during storage of cheese at 25 °C for 23 days. The number of Enterobacteriaceae decreased by 2.3 and 0.6 log, respectively, at 5, 15 and 25 °C (Fig. 2e).

**Survival of Salmonella enterica subsp. enterica in ripened raw milk cheese during storage**

The survival of Salmonella enterica subsp. enterica in the experimentally contaminated raw milk cheese during storage at 5, 15 and 25 °C is shown in Fig. 3. A decline in the number of microorganism was noticed reaching the final concentration level dependent on the applied storage temperature. The population of Salmonella enterica subsp. enterica from the initial level 6.45 log cfu g$^{-1}$ decreased by 2.2, 3.2 and 4.2 log cfu g$^{-1}$, respectively at 5, 15 and 25 °C. These findings corroborate with other studies where the behaviour of S. enterica subsp. enterica in the raw milk cheeses was analysed. In a study conducted by Stecchini et al. (1991), the growth of Salmonella Typhimurium was inhibited by L. plantarum due to its acid production during ripening of Montasio raw milk cheese. In our study, the number of Lactobacillus sp. was in the range of 6.5–8.0 log cfu g$^{-1}$. The activity of this microorganism could inhibit the growth of S. enterica subsp. enterica. Tamagnini et al. (2005) studied the behaviour of S. Typhimurium in Croston goat’s cheese. The author observed a reduction in the number of microorganism during storage by 1.0, 2.4 and 4.6 log cfu g$^{-1}$, respectively, at 5, 15 and 25 °C. Cheeses produced from raw milk contain a number of microbial communities, among which a representative subgroups of species are lactic acid bacteria (LAB). Leong et al. (2014) tested the ability of hard, semi-hard and soft market cheeses (67) produced from pasteurised milk to support growth, among others, of Salmonella spp. over 15 days at 25 °C. They reported that the pathogen did not grow on thirty cheeses, while fourteen cheeses supported growth of Salmonella spp. The cheeses that supported growth were characterised by pH in a range of 5.2–6.5, salt (%) – 1.0–3.0 and LAB number in most cases below 5 log cfu g$^{-1}$. In our study, the lactic acid bacteria concentration throughout the whole experiment was in a range of 6.5-8.0 log cfu g$^{-1}$. Native lactobacilli, lactococci and enterococci are able
to inhibit the growth of foodborne microorganisms by production of bacteriocins and other antimicrobial substances such as hydrogen peroxide and organic acids (Yoon et al., 2016).

The obtained survival curves of *S. enterica* subsp. *enterica* in the raw milk cheese were subjected to primary modelling. The kinetic parameters that characterise the behaviour of the *S. enterica* subsp. *enterica* estimated by Baranyi, Weibull and log-linear with tail models are presented in Table 1. The statistically significant differences (*P* < 0.05) were observed in case of $\mu_{\text{max}}$ estimated by the Baranyi model between 5, 15 and 25 °C, in case of $\delta$ estimated by the Weibull model among all studied temperatures, and in case of $k_{\text{max}}$ estimated by the log-linear model between 5, 15 and 25 °C (Table 1). The goodness of fit of the primary models was tested using the $F$-test (Table 2). The adequacy was satisfactory in case of the Weibull model at all applied storage temperatures, while the Baranyi model failed at 15 °C, and the log-linear with tail model failed at 5 °C. Moreover, the calculated statistical indices ($R^2$, $\text{MSE}_{\text{model}}$, AIC) for the fitted primary models confirmed that the Weibull model is more adequate in describing the behaviour of *S. enterica* subsp. *enterica* in the raw milk cheese (Table 2). Figure 3 shows fitted Weibull model for the observed growth of *S. enterica* subsp. *enterica* in the contaminated raw milk ripened cheese at 5, 15 and 25 °C.

The p parameter of the Weibull model has a biological interpretation. When p assumes values lower than...
Survival of Salmonella spp. in raw milk cheese 

Table 1 Kinetic parameters\(^a\) (means ± SD) of the Baranyi, Weibull and log-linear + tail models for the fate of S. enterica subsp. enterica in experimentally contaminated raw milk ripened cheese at 5, 15 and 25 °C

| Temperature (°C) | Baranyi | Weibull | Log-linear + tail |
|-----------------|---------|---------|-------------------|
|                 | \(N_0\) | \(N_{\text{max}}\) | \(\mu_{\text{max}}\) | \(\delta^*\) | \(\rho^*\) | \(k_{\text{max}}\) | \(N_0\) | \(N_{\text{res}}\) |
| 5               | 6.13 ± 0.06 | 4.45 ± 0.09 | −0.036 ± 0.003 | 17.13 ± 3.43 | 0.58 ± 0.06 | 6.42 ± 0.10 | 0.10 ± 0.01 | 6.17 ± 0.07 | 4.38 ± 0.11 |
| 15              | 6.40 ± 0.10 | 3.40 ± 0.02 | −0.083 ± 0.040 | 11.54 ± 1.50 | 0.73 ± 0.12 | 6.41 ± 0.10 | 0.17 ± 0.01 | 6.64 ± 0.07 | 3.48 ± 0.02 |
| 25              | 6.42 ± 0.05 | 2.30 ± 0.20 | −0.180 ± 0.005 | 6.32 ± 0.50 | 0.83 ± 0.05 | 6.58 ± 0.10 | 0.42 ± 0.01 | 6.43 ± 0.06 | 2.31 ± 0.18 |

\(N_0\) = initial cell concentration (log cfu g\(^{-1}\)), \(N_{\text{max}}\) = final cell concentration (log cfu g\(^{-1}\)), \(\mu_{\text{max}}\) = maximum specific growth rate (h\(^{-1}\)), \(\delta^*\) = Weibull parameter, \(\rho^*\) = Weibull parameter, \(k_{\text{max}}\) = maximum inactivation rate (d\(^{-1}\)), \(N_{\text{res}}\) = residual population density (log cfu g\(^{-1}\)).

Table 2 Statistical indices for the primary survival/inactivation models used to fit S. enterica subsp. enterica fate in experimentally contaminated raw milk ripened cheese at 5, 15 and 25 °C. Bold values indicate that the F-test was statistically acceptable.

| Temperature (°C) | Baranyi | Weibull | Log-linear + tail |
|-----------------|---------|---------|-------------------|
|                 | \(R^2\) | MSE\(_{\text{model}}\) | AIC | \(f\)-test | \(R^2\) | MSE\(_{\text{model}}\) | AIC | \(f\)-test | \(R^2\) | MSE\(_{\text{model}}\) | AIC | \(f\)-test |
| 5               | 0.87 | 0.04 | −197.32 | −200.33 | −176.78 | 0.63 | 0.97 | 2.15 | 0.93 | 0.07 | 0.03 | 0.07 | 2.65 | 1.20 | 1.35 |
| 15              | 0.96 | 0.05 | −148.91 | −185.17 | −161.92 | 0.96 | 0.07 | 0.96 | 0.96 | 0.07 | 0.02 | 0.06 | 3.35 | 1.20 | 1.35 |
| 25              | 0.30 | 0.81 | −161.94 | −180.59 | −170.78 | 0.30 | 0.81 | 0.80 | 0.30 | 0.81 | 0.80 | 0.30 | 0.81 | 0.80 |

Predictive microbiological models can be used by food manufacturers in the food product development processes, as well as a tool to support food safety assurance systems. Generated in this study predictive models provide precise predictions of Salmonella survival and support the quantitative microbiological risk assessment (QMRA) process in raw milk cheeses. The models can have practical application in the management of production, distribution and storage of raw milk cheese in terms of microbiological safety.

Figure 4 Impact of increased (+ 1 °C [---]) and decreased (−1 °C [---]) temperature on the fate of S. enterica subsp. enterica in raw milk cheese during storage at 5, 15 and 25 °C; [-] represents fitted model for studied temperatures.

Prediction of Salmonella enterica subsp. enterica survival at different storage temperatures

Nonlinear regression analysis was used in order to estimate the kinetic parameters of the secondary model that describe the survival of S. enterica subsp. enterica in raw milk cheese during storage at 5, 10 and 25 °C. Estimated values of \(z_{20^\circ C}\) and \(E_a\) were 13.64 ± 1.73 and −32.052.37 ± 266.449, respectively. Angelidis et al. (2013) showed that the inactivation rate increases proportionally to temperature when the value of \(E_a\) is negative. It can be assigned to the growth inhibition of...
$S.\ enterica$ subsp. $enterica$ in raw milk cheese as a result of lactic acid bacteria activity (Yoon, Lee, & Choi, 2016).

To study the relationship between the $\beta$ parameter of the Weibull model and the storage temperature ($K$), a linear regression was run resulting in the following equation: $\beta = 0.0125 \cdot T \cdot 2.89$. Figure 4 presents the effect of the kinetic parameters of the Weibull model and the impact of storage temperature variation on the fate of $S.\ enterica$ subsp. $enterica$ in raw milk cheese during storage at 5, 15 and 25 °C.

A reduction in the $S.\ enterica$ subsp. $enterica$ population by $2\ \log\ \text{cfu g}^{-1}$ was observed at 57, 30 and 16 days, respectively, at 5, 15 and 25 °C. The changes in the storage temperature of $+1\ °C$ and $-1\ °C$ from the initial temperature resulted in significant variation in the behaviour of the microorganism. A deviation of $+1\ °C$ caused a decrease of the reduction time in comparison with studied temperatures by 3, 2 and 1 h, respectively, at 5, 15 and 25 °C. Similarly, a deviation of $-1\ °C$ caused an increase of the reduction time in comparison with studied temperatures by 3, 2 and 1 h, respectively, at 5, 15 and 25 °C.

Conclusions

Due to the possible exposure of the consumer to the $S.\ enterica$ subsp. $enterica$ in dairy products made from raw milk, appropriate risk communication on the consumption of these products in particular to a vulnerable population is recommended. Moreover, the models presented in this study can be used in quantitative risk assessment studies to estimate the threat to consumers regarding the presence of $Salmonella$ in raw milk cheese.

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