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Modelling survival of *Salmonella* Enteritidis during storage of yoghurt at different temperatures

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ABSTRACT

The aim of this study was to evaluate the behaviour of *Salmonella* Enteritidis during the storage of yoghurt at different temperatures (4, 12, 20, and 25 °C), and to develop mathematical models to predict the behaviour of this bacterium as a function of storage temperature. Results indicated that *Salmonella* was able to survive longer during storage when temperature was low (e.g. 304 h at 4 °C, 60 h at 25 °C). The Geeraerd model with log-decrease and tailing was selected as the most suitable model to describe survival. To evaluate the effect of storage temperature on kinetic parameters such as death rate (k_{max}) secondary models were developed. The k_{max} was maximum at 25 °C and minimum at 4 °C with k_{max} = 0.28 and 0.039 h^{-1}, respectively. The residual population (N_{res}) ranged 0.5 and 1.8 log CFU/g but there was no temperature dependency of this parameter. A probabilistic example was conduced based on the developed model to assess the exposure to *Salmonella* by consumption of traditional Turkish yoghurt.

1. Introduction

Yoghurt is a traditional fermented dairy product obtained as a result of lactic acid fermentation by starter cultures, consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Due to its nutritional value and positive effect on health, it has an important role in the human diet (Hrnjez et al., 2014; Madhu et al., 2013; Sfakianakis and Tzia, 2014).

Although yoghurt is usually considered microbiologically safe (Ahmed et al., 2014; Reeta et al., 2015), there is some evidence that pathogens can grow and survive during the production of yoghurt in spite of the lactic acid and bacteriocins produced by yoghurt cultures (Massa et al., 1997; Szczawińska and Szczawiński, 2011). If hygiene practices are not adequate contamination by foodborne pathogens could occur during production of fermented milk products (Kumbhar et al., 2009) or subsequent handling during food preparation. In 1993, an outbreak of diarrhoea and HUS (haemolytic uraemic syndrome) occurred following the consumption of commercial yoghurt and *E. coli* O157:H7 was identified as the causative agent (Morgan et al., 1993). Although in a different context, the possible vulnerability of yoghurt to contamination by foodborne pathogens during production has been also highlighted by Food Drug Administration (FDA) in the study of Vulnerability Assessments of Food Systems (FDA, 2012).

Salmonella spp. is reported as a major cause of foodborne illness throughout the world and is also the most common cause of diseases associated with consumption of dairy products. (Scallan et al., 2011; CDC, 2011; Szczawińska and Szczawiński, 2011). The *Salmonella* genus has > 2500 serovars, and, in particular, *Salmonella* Enteritidis, the global predominant serovar, is a common cause of infections associated with various foods including dairy products (Rodriguez-Lazaro et al., 2014; Wang et al., 2016).

Although *Salmonella* cells have unfavourable conditions for growth in yoghurt, it may survive in the final product, depending on the type of the product, pH, storage temperature, and other environmental conditions (Szczawiński et al., 2014). The presence of *Salmonella* in the yoghurt production process may be a result of exceptionally highly contaminated raw milk (traditional process), inadequate heat treatment and recontamination due to a lack of hygiene during food packaging or handling (Szczawińska and Szczawiński, 2011).

Yoghurt is usually kept at around 4 °C during storage although other temperatures < 10 °C are also possible (Bachrouri et al., 2006). However, the product might be exposed to cold chain interruption for hours during industrial distribution, retailing and home storage (Ferdousi et al., 2013).

With the applications of predictive microbiology models, the behaviour of microorganisms under specific environmental factors can be

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estimated throughout the food supply chain (Pérez-Rodríguez and Valero, 2013). In this sense, predictions can be useful to develop or validate control measures. Although pathogen behaviour in yoghurt has been investigated by several research groups (Akkaya et al., 2009; Al-Haddad, 2003; Alvarez-Ordonez et al., 2013; Bachrouri et al., 2002; Canganella et al., 1999; Massa et al., 1997; Ogwaro et al., 2002; Pazakova et al., 1997), there are only few predictive model studies available in the literature (Bednarko-Młynarczyk et al., 2015; Szczawiński et al., 2014).

Therefore, the aim of this study was to evaluate the behaviour of *Salmonella* Enteritidis during the storage of traditional yoghurt at different temperatures (4, 12, 20, and 25 °C), and to develop the mathematical models able to predict the behaviour of this microorganism as a function of storage temperature.

2. Materials and methods

2.1. Strains and inoculum preparation

Salmonella enterica subsp. enterica serovar Enteritidis (ATCC 13076) and the traditional yoghurt cultures consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (the culture collection of the Food Microbiology Laboratory of Ankara University, Department of Food Engineering, Ankara, Turkey) were used.

Salmonella Enteritidis was cultured overnight in Tryptic Soy Broth (TSB, Merck 1.05459, Germany) at 37 °C, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were cultured overnight in M17 Broth (Merck 1.15029, Germany) and de Man, Rogosa and Sharpe Broth (MRS, Merck 1.10661, Germany), respectively, at 44 °C. Equal volumes of the starter cultures were mixed before use.

2.2. Yoghurt preparation and inoculation

The yoghurt samples were prepared under laboratory conditions by using 10% (w/v) skimmed milk solution (prepared from skimmed milk powder (Carl Roth, fat ≤ 1%)) following a typical static yoghurt production process. After sterilization (115 °C for 10 min.), 2 L milk were cooled up to 43–44 °C, and the yoghurt culture mixture (~9 log CFU/mL) was added at 2% (v/v) (~7 log CFU/mL). Simultaneously, 0.2 mL of overnight culture of *Salmonella* Enteritidis (~9 log CFU/mL) was added to obtain a *Salmonella* inoculum of 5 log CFU/mL aproximately in the milk prior to fermentation.

After stirring with a magnetic stirrer, 30 mL portions of the inoculated milk were distributed into sterile 100 mL - flasks and incubated at 44 °C for fermentation until the desired acidity was reached (pH 4.5) which was observed at 4.5 h.

Cooling of the coagulum was commenced directly after the product reached the desired acidity, by placing the flasks in a refrigerator at 4 °C overnight. Flasks were subsequently stored at 4, 12, 20, 25 °C in refrigerated incubators. Since the yoghurt microorganisms show limited growth activity around 10 °C, the primary objective of cooling was to drop the temperature of the coagulum from 30 to 45 °C to < 10 °C (best around 4 °C) as quickly as possible (Bachrouri et al., 2006). The experiments were replicated in three different days in order to capture the experimental variability.

2.3. Microbiological analysis

Yoghurt flasks were sampled from 10 to 19 times (separate flask each time) during storage, depending on the storage temperature. The sampling frequency corresponded to every 2 h at the beginning of the storage for the high temperatures (20 and 25 °C), and it was every 4–24 h for the low temperatures (4 and 12 °C). First, after mixing well of the sample, 5 g yoghurt was added to 45 mL Maximum Recovery Diluent (MRD, Merck 1.12535, Germany), and homogenized by shaking. (The rest of the sample was used for measuring of the pH). Then, 1 mL homogenized sample was transferred into a test tube containing 9 mL MRD and 10 fold serially diluted. The viable cells of *Salmonella* Enteritidis were determined by spreading 0.1 mL or pouring 1 mL of each dilution on Xylose Lysine Deoxycholate Agar (XLD, Merck 1.05287, Germany) (two plates per dilution) and by incubation at 37 °C for 24 h.

Yoghurt cultures were also analyzed at the beginning and at the end of the storage period. The *Streptococcus thermophilus* was enumerated on M17 agar (Merck 1.15108, Germany) spreading 0.1 mL on plate and incubating the plates aerobically at 44 °C for 24 h. The *Lactobacillus bulgaricus* was analyzed with MRS agar (Merck 1.10660, Germany), adjusted to pH 5.2 and incubated at 44 °C for 24 h by using duplicate pour plate method (overlayered the pour plates with approximate level of 5 mL sterile MRS agar).

When the *Salmonella* reached undetectable levels, one more determination was made for every storage temperature to assess the variable presence of residual population. The limit of detection (LOD) was 3 CFU/g (0.48 log CFU/g) based on the presence of one colony in 3 plates inoculated each with 1 mL of the dilution 1:10.

2.4. Measurement of pH

The pH of the samples was measured when the enumeration procedures were carried out. This was performed by inserting a probe electrode calibrated against buffer at pH 4.0 and 7.0, connected to a pH-meter (InoLab pH level 2), into well-mixed samples.

2.5. Development of predictive models

2.5.1. Modelling Salmonella survival over time at different temperature conditions

The *Salmonella* Enteritidis enumeration data obtained over time for all repetitions were transformed into $\log_{10}$ scale and pooled (all individual repetitions for each temperature were separately presented in graph to show real variability between experiments, not averaging them) for further analysis. Then, treated data were used to fit different primary survival models (Table 1) for each temperature by using the statistical package *nls* in R software (version 3.2.2) (https.//www.r-project.org/).

In order to evaluate goodness of fit of each model in Table 1, the root mean squared error (RMSE) and corrected Akaike Information Criterion (AIC_C) were calculated using Eqs. (1) and (2) and (3), respectively.

$$\mathrm{RMSE} = \sqrt{\frac{(\text{observed values} - \text{predicted values})^2}{n - \mathrm{k}}}. \quad (1)$$

where n is the number of observations and k, the number of model parameters.

$$AIC = 2k - 2\ln(L). \quad (2)$$

$$AIC_c = AIC + \frac{2k(k+1)}{n-k-1}. \quad (3)$$

where k is the number of model parameters, L is the maximized value of the likelihood function for the model and n is the number observations.

AIC_C determines the model with the fewest parameters that still provide an adequate fit to the data, and the lowest AIC_C value corresponds to the most adequate model. This parameter is particularly suitable for comparing non-nested models with a different number of parameters such as some of the models in this study (McKellar et al., 2014).

2.5.2. Modelling the effect of temperature on death rate of Salmonella

The kinetic parameters from the survival model were further analyzed as a function of storage temperature. The influence of temperature on the parameters (k_{max}) was evaluated mathematically by testing