Assessment of the *Cronobacter sakazakii* risk in reconstituted infant formula

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Abstract

The study focused on assessing *Cronobacter sakazakii* growth in reconstituted powdered infant formula at temperatures ranging from 5 °C to 48 °C using the Baranyi-Roberts model. The count of *C. sakazakii* was determined by the plate method on ESIA agar (44 °C, 24 h). Bacteria grew in reconstituted milk only at temperatures above 8 °C. The lag phase duration decreased with increasing temperature, from approximately 123.0–141.0 h at 8 °C to 0.931–1.281 h at 44 °C. The growth rate ranged from 0.025–0.027 ln cfu/ml/h (8 °C) to 2.441–2.633 ln cfu/ml/h (44 °C). The resulting growth models imply an increase of more than 4 orders of magnitude in the number of *C. sakazakii* in less than 17 h at 24 °C; at temperatures of 27 °C and above, the bacteria reach the critical concentration considered in our study (8 log cfu) in a few hours (4.5–11.9 h). In conclusion, it is unsafe and inappropriate to store reconstituted infant milk at temperatures higher than 8 °C.

Predictive microbiology, Baranyi-Roberts model, lag phase, growth rate

Bacteria of the genus *Cronobacter*, family *Enterobacteriaceae*, are Gram-negative facultatively anaerobic bacteria considered as potential foodborne pathogens (Li et al. 2024; Lindsay et al. 2024). They can cause necrotizing enterocolitis, meningitis and sepsis with severe sequelae, especially in newborns and infants (Cheng et al. 2024). Mortality can be as high as 40–80% (Csorba et al. 2021). At present, the genus *Cronobacter* includes seven species: *C. sakazakii*, *C. universalis, C. malonaticus*, *C. dublinensis*, *C. turicensis*, *C. muytjensii* and *C. condimenti* (Csorba et al. 2021; Lindsay et al. 2024).

Except for *C. condimenti* which has not been involved in any documented clinical episode, the other six species have clinical significance, with *C. sakazakii* and *C. malonaticus* representing pathogenic species of major public health concern, followed by *C. turicensis*, *C. universalis*, *C. muytjensii*, and *C. dublinensis* (Alsonosi et al. 2015). EFSA/ECDC (2023) reported the occurrence of *Cronobacter sakazakii* as defined by the Regulation (EC) No 2073/2005 to be 0.4% (two positive samples of dried infant formulae out of 452 collected samples from five member states).

Diseases caused by *Cronobacter* spp. in newborns and infants are mainly associated with the consumption of dried infant formula contaminated with this bacterium (Csorba et al. 2021). It is, therefore, very important to ensure increased control during production (Li et al. 2024). Contamination on dairy plants is likely to occur during fluid bed drying and spray drying (Csorba et al. 2021). The persistence of *Cronobacter* spp. in manufacturing environments is enhanced by its ability to form biofilms on stainless steel, polyvinyl, glass, and other surfaces (Li et al. 2024). In the form of a biofilm, the influence of the external

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environment on their growth is significantly reduced, which leads to better survival of the bacteria (Song et al. 2023). Another problem lies in the ability of *Cronobacter* spp. to survive in extreme conditions (drought, heat, acidic environments), such as in foods with low moisture content (powdered milk, baby food, cereals, flour, or spices). In such foods, these bacteria can survive for more than two years (Cheng et al. 2024; Li et al. 2024).

The World Health Organization has specific instructions for the preparation of powdered infant formula in care settings and home settings (WHO 2007). This document emphasizes the necessity of washing hands before preparation and boiling the bottles before use. The boiled water used for milk reconstitution needs to be cooled slightly but not under 70 °C. Mixing bottles can be shaken to fully mix the water and powdered infant formula, feeding cups can be stirred with a pre-sterilized spoon. Microwaving should not be used when preparing powdered infant formula. Leftover feed is suggested to be thrown away and, in particular, it should not be used if stored for more than 2 h at room temperature. However, in a sealed container in the refrigerator, it can be stored for up to 24 h.

To ensure food safety, it is crucial to prevent the growth of undesirable microorganisms or to destroy them. Predictive mathematical modelling can be used to predict microorganism behaviour depending on factors such as temperature, moisture, water activity, or other storage conditions and thus, to evaluate the entire process of food preparation and decide on the microbiological safety of a given food under specific conditions (Stavropoulou and Bezirtzoglou 2019).

Yoon et al. (2019) used predictive models to determine fundamental kinetic parameters that describe characteristic behaviors of these bacteria under dynamic environments and evaluated the thermal resistances of *Cronobacter sakazakii*. Jo et al. (2010) developed a mathematical model to predict the growth rate of *C. sakazakii* in infant milk based on temperature; Wesseling et al. (2019) developed predictive models for the growth of *C. sakazakii* not only in infant milk formula but also in infant soy formula.

The presented study aimed to evaluate the effect of storage temperature on the kinetic parameters of *C. sakazakii* growth in reconstituted infant milk. The growth dynamics were evaluated using the Baranyi-Roberts model. The developed growth model was used to estimate the potential risk to consumers.

Materials and Methods

Bacterial strains

To evaluate the growth dynamics of *Cronobacter sakazakii* in reconstituted infant formula milk, multiple strains of *C. sakazakii* available from collections of microorganisms were used. Table 1 gives an overview of the strains used and their origin.

Infant formula reconstitution, inoculation, and storage

Before the start of the experiments, 25 g of milk powder were tested for the presence of *C. sakazakii* according to ČSN EN ISO 22964 (2017) with a negative result.

The effect of temperature on the growth dynamics of *Cronobacter sakazakii* was investigated in the reconstituted Sunar® complex 1 infant formula (Hero, Lenzburg, Switzerland). A mixture of three collection strains of *C. sakazakii* was used to inoculate the samples (see Table 1). For inoculation, individual strains were cultured

Table 1. Used *Cronobacter sakazakii* strains and their origin.

Strain	Origin (sample)	Collection
Cronobacter sakazakii 372 (serotype O:4)	cucumber	VRI
Cronobacter sakazakii 484 (serotype O:2)	carrot	VRI
Cronobacter sakazakii CCM 1902	reference strain	CCM

VRI: Collection of the Veterinary Research Institute (Brno, Czech Republic); CCM: Czech Collection of Microorganisms (Brno, Czech Republic)

aerobically on blood agar (Bio-Rad, Marnes-la-Coquette, France) at 37 °C for 24 h. Subsequently, a suspension in sterile saline solution with a density of about 8 log cfu/ml was prepared for each strain, and the individual suspensions were mixed in the same proportion. The resulting mixed suspension was diluted as required and used to inoculate the milk powder.

An appropriate amount of the mixed suspension was added to 7.17 g of milk powder in a sterile bottle. The inoculated milk powder was then reconstituted with 50 ml of boiled drinking water cooled to the required temperature according to the manufacturer's instructions. In this way, 3 parallel samples of reconstituted milk with an initial concentration in the order of 1–2 log cfu/ml were prepared for each investigated temperature. Reconstituted milk without the addition of *C. sakazakii* was used as the negative control. The inoculated reconstituted milk was stored at temperatures of 5, 8, 12, 15, 18, 21, 24, 27, 30, 37, 44, and 48 °C. The length of storage varied according to the temperature used, namely 14 days at 5–12 °C, 96 h at 15–30 °C, 48 h at 37–44 °C, and 31 h at 48 °C. The temperature range was chosen to capture the minimum and maximum growth temperature of the bacterium. The storage time was then taken into account to achieve complete growth curves, i.e., to capture all the basic growth phases of bacterial population: the lag phase, the exponential phase, and the stationary growth phase.

Quantitative detection of *Cronobacter sakazakii*

Samples of reconstituted infant formula for *C. sakazakii* enumeration were taken aseptically after a thorough mixing of the milk. Individual samples were examined immediately after inoculation (0 h) and at 0.5, 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, and 14 days (temperatures 5–12 °C); 3, 6, 9, 12, 24, 31, 48, 56, 72, and 96 h (temperatures 15–30 °C); 1, 2, 3, 4, 6, 8, 10, 12, 24, 31, and 48 h (temperatures 37–44 °C); and 1, 2, 3, 4, 6, 8, 10, 12, 24, and 31 h (temperature 48 °C).

Basic processing of the collected samples was performed in accordance with ČSN EN ISO 6887-1 (2018). Sterile saline solution enriched with peptone (0.85% NaCl + 0.1% peptone, pH 7) was used as a dilution medium.

The count was determined on chromogenic Modified HiCrome *Ent. sakazakii* agar (ESIA; HiMedia Laboratories Pvt. Ltd., Mumbai, India). The Petri dishes were incubated aerobically at 44 °C for 24 h. For each temperature tested, the experiment was carried out in triplicates with an additional negative control.

Statistical analysis

All the data (cfu/ml) were logarithmically transformed to the log 10 scale, and the mean values and standard deviations were calculated. *Cronobacter sakazakii* dynamics were examined using a primary mathematical model for microbial growth (the change in bacterial number over time). Data were fitted to the Baranyi-Roberts model (Baranyi and Roberts 1994), with the following parameters chosen to interpret the model: growth rate μ_{max}, lag phase duration λ, and maximum number of microorganisms. The generated growth model was also used to calculate the time needed for *C. sakazakii* to achieve a critical concentration causing poisoning in humans. The R software, version 3.4.3, and the nlsMicrobio library (Baty et al. 2014) were used to model the relationships.

Results

Table 2. Mean ± standard deviation of the number of *Cronobacter sakazakii* (log cfu/ml) in reconstituted infant milk stored for 14 days at 5 °C, 8 °C and 12 °C (n = 3).

Duration (days)	Storage temperature		
	5 °C	8 °C	12 °C
0	2.05 ± 0.02	2.23 ± 0.08	2.23 ± 0.10
0.5	2.00 ± 0.07	2.20 ± 0.10	2.35 ± 0.02
1	1.98 ± 0.07	2.19 ± 0.10	2.81 ± 0.05
2	1.72 ± 0.06	2.31 ± 0.06	3.84 ± 0.20
3	1.60 ± 0.09	2.33 ± 0.03	5.30 ± 0.14
4	1.43 ± 0.03	2.38 ± 0.04	6.80 ± 0.14
6	1.38 ± 0.18	2.47 ± 0.02	8.33 ± 0.19
7	1.41 ± 0.12	2.71 ± 0.07	8.46 ± 0.15
8	1.06 ± 0.10	2.94 ± 0.09	8.40 ± 0.04
9	1.10 ± 0.35	3.35 ± 0.30	8.39 ± 0.05
10	1.01 ± 0.10	3.52 ± 0.05	8.30 ± 0.07
11	0.97 ± 0.18	3.81 ± 0.28	8.31 ± 0.14
13	0.86 ± 0.05	4.32 ± 0.23	8.17 ± 0.16
14	1.13 ± 0.21	4.46 ± 0.28	8.10 ± 0.07

The mean *C. sakazakii* counts detected for each storage temperature are presented in Tables 2–4. The *C. sakazakii* count did not exceed the detection limit of the used plate method 0.69 log cfu/g in any of the control samples (milk powder without added *C. sakazakii*). In other words, there was no growth of typical colonies after smearing 0.2 ml of undiluted control sample.

The created growth curves of *C. sakazakii* for the investigated temperatures are shown in Fig. 1 and the parameters of the created models in Table 5.

At a temperature of 5 °C, no growth of *C. sakazakii* was observed during 14 days of storage; there was even a slight

Table 3. Mean ± standard deviation of the number of *Cronobacter sakazakii* (log cfu/ml) in reconstituted infant milk stored for 96 h at 15–30 °C (n = 3).

Duration (h)	Storage temperature					
	15 °C	18 °C	21 °C	24 °C	27 °C	30 °C
0	1.93 ± 0.04	2.07 ± 0.04	1.94 ± 0.10	1.96 ± 0.01	2.17 ± 0.09	1.91 ± 0.05
3	1.90 ± 0.03	2.06 ± 0.06	1.96 ± 0.01	2.12 ± 0.05	2.61 ± 0.09	2.65 ± 0.06
6	1.86 ± 0.07	2.33 ± 0.03	2.64 ± 0.03	2.68 ± 0.15	3.69 ± 0.04	4.04 ± 0.00
9	2.05 ± 0.02	2.86 ± 0.03	3.36 ± 0.02	3.55 ± 0.07	5.67 ± 0.15	5.66 ± 0.05
12	2.27 ± 0.06	3.21 ± 0.07	3.85 ± 0.13	4.32 ± 0.16	6.94 ± 0.12	7.06 ± 0.09
24	3.60 ± 0.01	5.22 ± 0.29	6.52 ± 0.06	8.40 ± 0.02	9.30 ± 0.09	9.72 ± 0.41
31	3.98 ± 0.09	5.98 ± 0.06	7.94 ± 0.21	9.06 ± 0.44	9.50 ± 0.05	9.64 ± 0.06
48	5.63 ± 0.15	8.57 ± 0.06	9.13 ± 0.11	9.49 ± 0.06	9.28 ± 0.13	9.74 ± 0.27
56	6.42 ± 0.35	8.87 ± 0.15	9.13 ± 0.11	9.49 ± 0.10	9.65 ± 0.19	9.88 ± 0.26
72	8.07 ± 0.02	9.12 ± 0.11	9.07 ± 0.08	9.60 ± 0.14	9.65 ± 0.17	9.77 ± 0.12
96	8.68 ± 0.02	9.27 ± 0.04	9.35 ± 0.15	9.79 ± 0.21	10.00 ± 0.16	9.60 ± 0.30

Table 4. Mean ± standard deviation of the number of *Cronobacter sakazakii* (log cfu/ml) in reconstituted infant milk stored for 48 h at 37 °C, 44 °C and 48 °C (n = 3).

Duration (h)	Storage temperature		
	37 °C	44 °C	48 °C
0	2.19 ± 0.04	2.26 ± 0.05	2.30 ± 0.02
1	2.25 ± 0.07	2.39 ± 0.03	2.35 ± 0.05
2	2.61 ± 0.21	3.15 ± 0.07	2.81 ± 0.05
3	3.48 ± 0.04	4.34 ± 0.02	3.44 ± 0.08
4	4.62 ± 0.07	5.50 ± 0.04	4.08 ± 0.20
6	6.80 ± 0.04	7.66 ± 0.03	4.93 ± 0.13
8	8.08 ± 0.17	8.70 ± 0.09	5.94 ± 0.10
10	8.82 ± 0.03	9.19 ± 0.24	6.64 ± 0.07
12	9.22 ± 0.09	9.53 ± 0.09	6.98 ± 0.29
24	9.91 ± 0.04	9.36 ± 0.24	7.06 ± 0.32
31	10.12 ± 0.22	9.29 ± 0.01	6.35 ± 0.16
48	9.83 ± 0.10	8.94 ± 0.13	ND

ND: determination not performed

decrease in the number of bacteria (by approximately an order of magnitude). A linear model of this growth is shown in Fig. 1. This finding implies that the minimum growth temperature of *C. sakazakii* is > 5 °C. This is in accordance with the results reported by Fang et al. (2012) who observed no *C. sakazakii* growth in reconstituted powdered infant formula at 6 °C for 11 days and stated the minimum growth temperature for the tested *C. sakazakii* strains to be higher than 6 °C.

As can be seen from Fig. 1, the number of bacteria slightly increased at 8 °C, although the complete growth curve was not produced within 14 days (i.e., the stationary growth phase was not reached). At 12 °C, the growth curve produced within 14 days was complete and the growth of the bacterial population is shown (Fig. 1). The maximum number of *C. sakazakii* at this temperature reached 8 log cfu/ml (Table 5, Fig. 1).

Unsurprisingly, complete growth curves were acquired also at higher storage temperatures (15–30 °C). Interestingly, starting at 27 °C, the *C. sakazakii* population in reconstituted infant milk already reached the stationary growth phase in less than 24 h (Fig. 1). At 37 °C and 44 °C, the growth curves were also complete, characterized by a very short lag phase (1–1.5 h) and a rapid onset of the stationary phase (approximately 8 h) (Fig. 1). At storage temperatures of 15–44 °C, maximum *C. sakazakii* counts were determined to be in the range of approximately 9–10 log cfu/ml (Tables 3 and 4). At 48 °C, *C. sakazakii* still proliferated, but the maximum counts were almost 3 orders of magnitude lower than at the previous temperatures (Fig. 1, Table 4). It is clear from the growth curve that this chosen temperature was already very close to the maximum growth temperature of the bacterium.

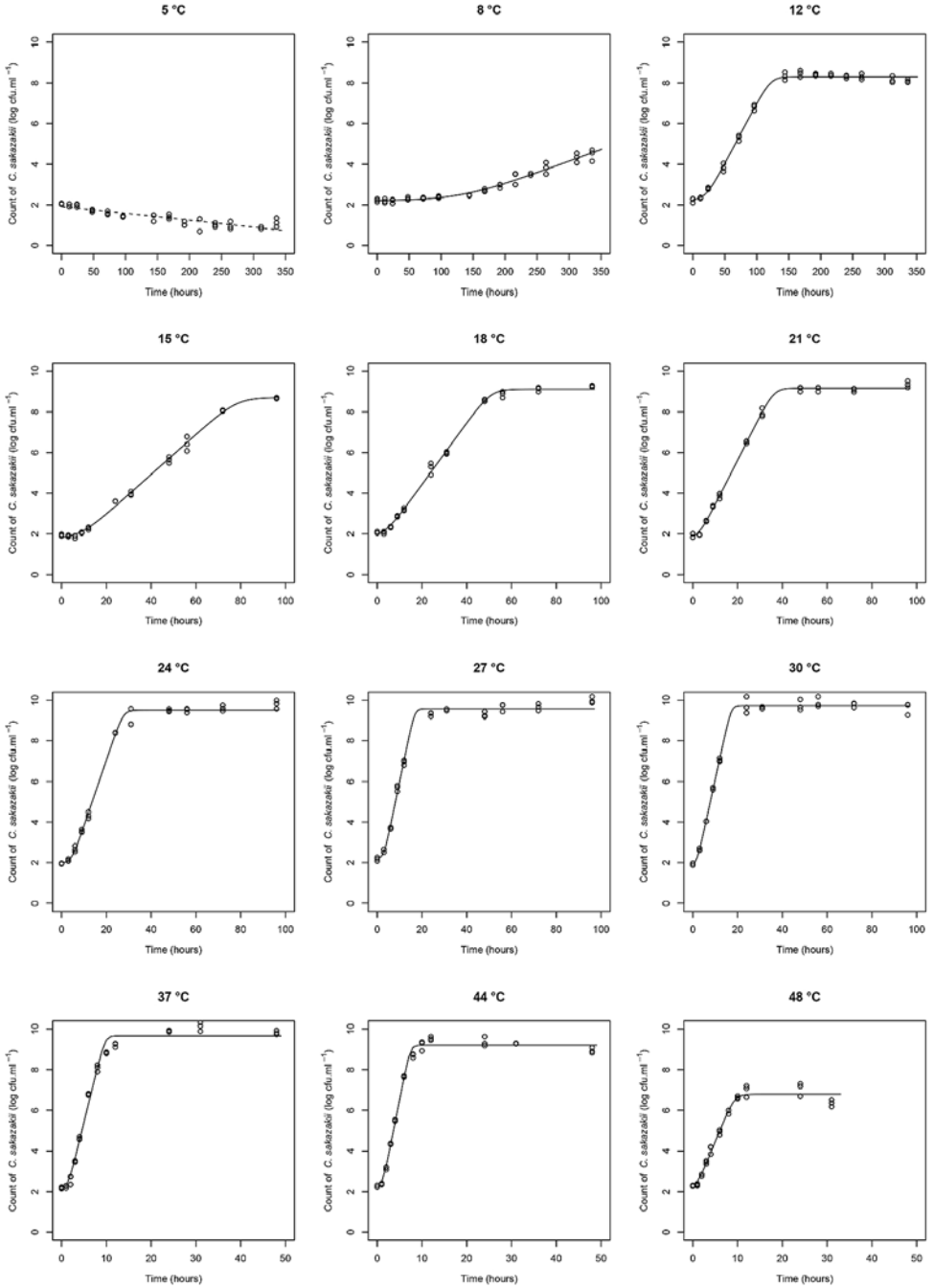

Fig. 1. Growth curves of *Cronobacter sakazakii* in reconstituted infant formula stored at 5 °C to 48 °C. Determined values (symbols) and predicted models (dash line: linear model for *Cronobacter sakazakii* at 5 °C, solid line: Baranyi-Roberts model for *Cronobacter sakazakii* growth at 12–48 °C). Initial concentration of *Cronobacter sakazakii* 1–2 log cfu/ml.

Table 5. The Baranyi-Roberts growth models of *Cronobacter sakazakii* in reconstituted infant formula at different temperatures. Initial count approximately 1–2 log cfu/ml. Estimate ± standard error of parameter.

Temperature	Lag phase duration λ (h)	Growth rate μ_{max} (ln cfu/ml/h)	Initial count (log cfu/ml)	Final count (log cfu/ml)	RSE	R^2
8 °C	132.0 ± 9.016	0.026 ± 0.001	2.218 ± 0.040	–[a]	0.149	0.967
12 °C	20.144 ± 2.441	0.137 ± 0.005	2.262 ± 0.068	8.310 ± 0.030	0.147	0.997
15 °C	8.528 ± 1.178	0.227 ± 0.005	1.823 ± 0.059	8.701 ± 0.089	0.152	0.997
18 °C	3.881 ± 0.799	0.350 ± 0.008	1.977 ± 0.074	9.118 ± 0.054	0.152	0.998
21 °C	2.712 ± 0.518	0.501 ± 0.009	1.854 ± 0.073	9.168 ± 0.040	0.140	0.998
24 °C	4.329 ± 0.559	0.745 ± 0.025	1.979 ± 0.108	9.502 ± 0.060	0.229	0.996
27 °C	2.872 ± 0.476	1.218 ± 0.065	2.205 ± 0.133	9.565 ± 0.057	0.244	0.994
30 °C	1.691 ± 0.358	1.157 ± 0.042	1.914 ± 0.109	9.726 ± 0.045	0.190	0.996
37 °C	1.317 ± 0.313	2.057 ± 0.097	2.123 ± 0.172	9.671 ± 0.099	0.353	0.988
44 °C	1.106 ± 0.175	2.537 ± 0.096	2.217 ± 0.113	9.197 ± 0.054	0.220	0.995
48 °C	0.872 ± 0.407	1.244 ± 0.075	2.217 ± 0.128	6.796 ± 0.078	0.243	0.984

[a] Incomplete growth model without a stationary phase; RSE: residual standard error; R^2: correlation between observed values and values predicted by the model

Table 6. Storage time to increase *Cronobacter sakazakii* to critical levels in one serving of reconstituted infant formula (100 ml, 50 ml, and 10 ml) inoculated to a starting concentration of around 1–2 log cfu/ml. Storage time (hours) predicted by the Baranyi-Roberts model.

Temperature	Time (hours) necessary to reach the critical concentration *C. sakazakii*		
	8 log cfu in 10 ml (i.e. 7 log cfu/ml)	8 log cfu in 50 ml (i.e. 6.3 log cfu/ml)	8 log cfu in 100 ml (i.e. 6 log cfu/ml)
8 °C	> 336[a]	> 336[a]	> 336[a]
12 °C	103.8	88.3	83.2
15 °C	61.4	54.0	51.0
18 °C	37.3	32.4	30.4
21 °C	26.4	23.1	21.8
24 °C	19.9	17.7	16.8
27 °C	11.9	10.6	10.0
30 °C	11.8	10.4	9.8
37 °C	6.8	6.0	5.7
44 °C	5.5	4.8	4.5
48 °C	–[b]	9.0	8.0

[a] Critical concentration was not reached during the 14 days of the experiment; [b] at 48 °C the final count of *C. sakazakii* was less than 7 log cfu/ml

Based on the evaluation of selected parameters of the models (residual standard error RSE, the correlation between observed values and values predicted by the model, see Table 5), it can be summarized that the Baranyi-Roberts very well describes the growth of *C. sakazakii* in reconstituted infant milk.

Our results also show that the duration of the lag phase was affected by storage temperature. The lag phase duration decreased with increasing temperature (Table 5). At high storage temperatures, the lag phase lasted for 1.0–1.5 h on average. The maximum growth rate of the bacterial population was also negatively associated with the duration of the lag phase (Table 5).

Similar to the lag phase duration, the growth rate was also significantly affected by storage temperature. The maximum growth rate of the bacterial population also increased with increasing temperature (Table 5).

The exact infectious dose of *Cronobacter sakazakii* has not been determined but is estimated to be at least 3 log cfu per gram or ml of food. According to the World Health Organization, the estimated oral infectious dose for newborns is between 3–8 log cfu (Pagotto and Abdesselam 2013). Given that the baseline concentration of *C. sakazakii* in reconstituted infant milk was in the range of 1–2 log cfu/ml, only the highest estimated infectious dose, i.e., 8 log cfu, was considered in estimating the time required for the bacterium to multiply to a critical level capable of inducing alimentary disease in three different portions of reconstituted milk – 10 ml, 50 ml, and 100 ml (see Table 6). The Baranyi-Roberts model was used for estimation and showed good agreement between observed and predicted values over the whole range of temperatures observed.

Discussion

Fang et al. (2012) compared the applicability of three primary mathematical models – the Baranyi-Roberts, Huang, and logistic models – to predicting *C. sakazakii* growth. The authors reported that all three models are useful for predicting the growth of *C. sakazakii* in infant milk. The results of a similar study by Wesseling et al. (2019) show that the Baranyi-Roberts, Gompertz, and logistic models are all able to predict the growth of *C. sakazakii* in reconstituted human milk very well. In all cases, a very good agreement was observed between the observed and model-predicted values, with the results obtained by the Gompertz model being ranked as the least accurate. On the other hand, the Baranyi-Roberts model was assessed as suitable for the prediction of *C. sakazakii* growth.

A significant effect of temperature on the duration of the lag phase and the growth rate of the bacterial population was confirmed in other studies as well (Jo et al. 2010; Fang et al. 2012; Fakruddin et al. 2016). Wesseling et al. (2019) reported the lag phase predicted by Baranyi-Roberts model to be 9.43 h at 15 °C and 2.79 h at 20 °C, which is consistent with our results. At higher temperatures between 28 °C and 47 °C, the predicted lag phase was always under 1 h. The longer duration of the lag phase in our study can be explained by the use of different *C. sakazakii* strains, amount of the inoculated milk (10 ml in a screw cap test tube in their study vs 50 ml in screw cap glass bottle in ours), incubation method (water bath in their study, incubator in ours) and, last but not least, the method of *C. sakazakii* count determination (addition of Tween-80 and VRBG agar in their study, no addition of Tween-80 and a chromogenic ESIA agar in ours).

Using the Gompertz primary model, Jo et al. (2010) determined the specific growth rate of *C. sakazakii* in reconstituted infant milk at temperatures ranging from 10 °C to 40 °C to be between 0.0263 ± 0.0007 log cfu/ml/h and 0.8058 ± 0.0395 log cfu/ml/h (growth rate increased with increasing temperature). Similar values were also reported by Fakruddin et al. (2016), namely 0.0251 ± 0.0011 log cfu/ml/h to 0.7861 ± 0.0027 log cfu/ml/h. This is consistent with our results, where at the corresponding temperatures (12–44 °C), the growth rate predicted by the Baranyi-Roberts model ranged from 0.132–0.142 ln cfu/ml/h (i.e., 0.057–0.062 log cfu/ml/h) at 12 °C to 2.441–2.633 ln cfu/ml/h (i.e., 1.060–1.144 log cfu/ml/h) at 44 °C. In the cited studies, the stationary growth phase was not reached in most cases. From this point of view, the (Baranyi-Roberts) growth models developed by us can therefore be considered more accurate.

The limit value for *Cronobacter* spp. in dried infant formulae and dried dietary foods for special medical purposes intended for infants under 6 months of age is given as the absence of the bacterium in 10 g of food (Commission Regulation (EC) 2073/2005).

In practice, this means that the presence of a single viable *Cronobacter* spp. cell in 10 g of product renders the product non-compliant. Havelaar and Zwietering (2004) reported that contamination of 18 g of infant milk powder (amount needed for one portion of reconstituted milk) with a single *Cronobacter* spp. cell caused bacterial mutiplication to an infectious dose of 3 log cfu at room temperature of 21 °C within 13 h.

In both these aforementioned studies, an increase of approximately three orders of magnitude in the number of *C. sakazakii* is considered to present risk to infant health. From our predicted values (Table 6), it is clear that at a normal room temperature of 24 °C, there will be an increase of more than 4 orders of magnitude in the number of *C. sakazakii* in less than 17 h, which is essentially consistent with their results. It is also clear from the results in Table 6 that at temperatures of 27 °C and above, the bacteria multiply to the highest considered critical concentration in a few hours (4.5–11.9 h). In the case of the minimum considered infectious dose of 3 log cfu in a portion of milk, this time would be even shorter. Therefore, storage of reconstituted breast milk at room temperature is clearly associated with a risk of alimentary infection caused by *Cronobacter* spp.

In conclusion, knowledge of the growth dynamics of pathogenic bacteria and its dependence on storage temperature is indispensable for the evaluation of food safety. The Baranyi-Roberts model well characterized the growth dynamics of *Cronobacter sakazakii* in reconstituted powdered infant formula. Based on the prediction of the time required for *C. sakazakii* to multiply to critical levels capable of inducing alimentary disease, it can be concluded that storage of reconstituted infant formula at room temperature is clearly associated with the risk of alimentary infection caused by *Cronobacter* spp. For safety reasons, reconstituted milk should be consumed as soon as possible or stored at temperatures below 8 °C for a short time.

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