Time-temperature profiles of infant milk formula in hospitals and analysis of Enterobacter sakazakii growth

Philippe Rosset, Véronique Noel, Elisabeth Morelli

TIME-TEMPERATURE PROFILES OF INFANT MILK FORMULA IN HOSPITALS AND ANALYSIS OF *ENTEROBACTER SAKAZAKII* GROWTH

P. ROSSET (*), V. NOEL, E. MORELLI

Agence Française de Sécurité Sanitaire des Aliments, LERQAP,
23 av. du Général de Gaulle, 94709 Maisons-Alfort cedex,France

Abstract

The purpose of this study was to assess the temperature conditions in neonatal care units for the preparation and storage of infant milk formula (IMF) and infant feeding using bottles and continuous feeding syringes. *Enterobacter sakazakii* in IMF for feeding infants has been chosen as the subject of this study because of the high risk incurred by IMF manufacture without total microbial destruction and the high sensitivity and mortality rates of this population group.

From IMF preparation till neonate feeding, time-temperature profiles of IMF samples were monitored and analysed. In order to show the health impact of this data, potential *E. sakazakii* growth was calculated. As IMF can be also contaminated with *Salmonella*, potential *Salmonella* growth was also calculated. However potential *Enterobacter sakazakii* growth data were only analyzed because of *Enterobacter sakazakii* and *Salmonella spp* data being close.

The study of 25 neonatal care units in 15 hospitals showed that the final potential growth for bottles depended on different parameters: initial water temperature, room temperature where IMF was prepared, cold storage temperature and time, reheating temperature and time. One parameter was not usually enough to determine the final growth increment alone and a well controlled and high performance stage could result in an incorrect food safety indication if the other stages are less effective.

On the other hand, the final potential growth for the continuous feeding syringes depended mainly on the feeding period since the IMF was kept in a particularly high ambient air temperature (≈ 25°C) in the infant's bedroom. This stage would be controlled first (with a cold syringe cover for example); then, as for bottles, the other stages would be controlled to result in a correct food safety indication.

Keywords: Risk factors; Predictive microbiology; infant milk formula, *Enterobacter sakazakii*

Introduction

Enterobacter sakazakii in infant milk formula (IMF) has been implicated in infant infections, especially among high-risk infants who are premature, have a low birth weight or are immunocompromised. The overall incidence of disease due to *Enterobacter sakazakii* is relatively low, with about 50 cases among 60 days old infants reported worldwide over the last 40 years (Iversen, Forsythe, 2003; Lehner, Stephan, 2004). Data on these infants indicated most of them were pre-terme, below 37 weeks gestation, and had a low birth-weight, below 2500 g, or peripartum complications (Lai, 2001). Mortality rates of 40 to 80% (Nazarowec-White, Farber, 1997a; Edelson-Mammel, Buchanan, 2004) have been reported. Survivors often suffer from serious brain damage such as hydrocephalus, quadriplegia and late neural development. Up until now, due to the limited information available on *E. sakazakii* exposure, the dose-response curve, probably with a linear relationship (Lehner, Stephan, 2004), has not been developed.

* Author's details: Fax +33.143.689.762. *E-mail address*: p.rosset@afssa.fr

When breastfeeding is not possible, infants are fed with infant formula containing protein from cow's milk or soja. The nutritional composition of these products complies with the standards defined in directives (Commission Directives 91/321/EEC, 96/4/EC, 199/50/EC and 1999/21/EC)

Provided by manufacturers, these products are ready-to-use in liquid or powder forms. While all liquid forms are sterile, the microbial (mainly *Enterobacter sakazakii*, *Salmonella*) destruction is never total in milk powder forms. However, none of these sterile forms are used for feeding high-risk infants because they have to be adjusted according to nutritional and/or medical individual prescriptions.

Because IMF is not sterile, good hygienic practices (i.e. rehydratation, cold storage and reheating) for its preparation and distribution to infants are crucial in order to prevent secondary contamination and multiplication. Public health officials such as the World Health Organization (WHO), and French Food Safety Agency (AFSSA) provide recommendations (WHO, 2004; AFSSA, 2005) for the use of IMF. Recent outbreaks due to *Enterobacter sakazakii* in IMF - 9 cases in 4 different hospitals, 2 seriously ill and 2 deaths in October-December 2004 in France (InVS, 2004; French Ministry for Solidarity, Health and the Family, 2004) – again highlight the extent to which these potential health risks must be controlled.

The purpose of this study was to assess temperature conditions in hospitals for the preparation and storage of IMF and feeding it to infants. In order to show the health impact of IMF, *Enterobacter sakazakii* and *Salmonella spp* growths were predicted using standard predictive microbiology models (van Gerwen, Zwietering, 1998).

Materials and methods

Sampling area and materials

This study was conducted in 25 neonatal care units of 15 public hospitals located in Paris (6) and its suburbs (9).

Two different feeding methods were studied according to oral or enteral nutrition. In the case of oral nutrition, IMF is poured into bottles, stored in a cold cabinet, reheated and consumed. In the case of enteral nutrition instead of (or in addition to) oral nutrition, IMF is collected in a syringe and stored in a cold cabinet until consumption. For feeding, the syringe is placed in an automatic continuous enteral nutrition system and connected with enteral tubing to the nasogastric tube. This system remains in place at room temperature for several hours (≤ 3 hrs.). All these studies were on powdered IMF.

According to the French Public Health Code (Decree No. 98-899, 1998), all of the health establishments studied have a specific room intended for IMF reconstitution. In all the healthcare establishments studied, bottles and feeding syringes were prepared in advance, in the morning, for a consumption period of 24 hours. As infants are generally fed every 3 hours, the subsequent 24 hours could be divided up into 8 feeding periods.

Temperature measurement

Temperature was measured by introducing time temperature indicators (TTIs) (Proges Plus, Willems, France) into reconstituted powdered infant formula. Temperature was monitored throughout the preparation and distribution stages: preparation, cold storage, possible transportation

to a neonatal department, feeding. Measuring started as soon as the first bottle/syringe was prepared. Both feeding methods (bottle, continuous feeding syringe) were studied. For each, IFM was studied in the best and the worst conditions. For feeding bottles, the best conditions were the first feeding period and the worst were the last feeding period. For feeding syringes, the best conditions were the first feeding period and a feeding during one hour; the worst were the last feeding period and a feeding during three hours.

Model for calculation of the potential microbial growth

Modeling was based on simple and standard predictive microbiology models. The chosen primary model is the exponential growth, without lag phase or stationary phase (fail-safe choice):

(A) $$N_{i+1} = N_i \exp(\mu_i \, t_i) \Leftrightarrow \ln\left(\frac{N_{i+1}}{N_i}\right) = \mu_i \, t_i$$

where N_i (resp. N_{i+1}) is the number of microorganisms (CFU/g) at time i (resp i+1), μ_i is the growth rate ($hour^{-1}$) assumed constant between i and i+1, and t_i is the timeframe (hours) between i and i+1.

Thus, for each timeframe between i and i+1, the microbial population N is multiplied by $\exp(\mu_i t_i)$ and the growth increment during this period is $N_{i+1} - N_i$. This relation can be also expressed in decimal logarithm, which is easier to interpret:

(B) $$\log(N_{i+1}) - \log(N_i) = \frac{\mu_i \, t_i}{\ln(10)}$$

The chosen secondary model is a cardinal temperature model with inflection (CTMI) (Rosso, 1993):

(C) $$\begin{cases} \text{if } T_i \le T_{min} \text{ or if } T_i \ge T_{max} & \mu(T_i) = 0 \\ \text{if } T_{min} < T_i < T_{max} & \mu(T_i) = \dfrac{\mu_{opt}\,(T_i - T_{max})(T_i - T_{min})^2}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T_i - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T_i)\right]} \end{cases}$$

where T_i is the temperature (°C) assumed constant between i et i+1 ; T_{min} is the theorical minimal temperature (°C) of the species ; T_{max} is the theorical maximal temperature (°C) of the species ; T_{opt} is the theorical optimal temperature (°C) of the species ; μ_{opt} is the growth rate ($hour^{-1}$) of the species in the food at temperature T_{opt}

From (A) and (C), the logarithm microbial increment for each timeframe is:

(D) $$\log(N_{i+1}) - \log(N_i) = \frac{t_i}{\ln(10)} \times \frac{\mu_{opt}\,(T_i - T_{max})(T_i - T_{min})^2}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T_i - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T_i)\right]}$$

where N_i (resp. N_{i+1}) is the number of microorganisms (CFU/g) at time i (resp i+1) ; t_i is the time duration (hours) between i and i+1 ; T_i is the temperature (°C) assumed constant between i et i+1 ; T_{min} is the theorical minimal temperature (°C) of the species ; T_{max} is the theorical maximal temperature (°C) of the species ; T_{opt} is the theorical optimal temperature (°C) of the species ; μ_{opt} is the growth rate ($hour^{-1}$) of the species in the food at temperature T_{opt}

For n successive steps, the total growth increment is:

$$\text{(E)} \qquad \Delta \log = \frac{\mu_{opt}}{\ln(10)} \times \sum_{i=1}^{n} t_i \frac{(T_i - T_{max})(T_i - T_{min})^2}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T_i - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T_i)\right]}$$

where t_i is the timeframe (hours) between i and i+1; T_i is the temperature (°C) assumed constant between i and i+1; T_{min} is the theoretical minimum temperature (°C) of the species; T_{max} is the theoretical maximum temperature (°C) of the species; T_{opt} is the theoretical optimum temperature (°C) of the species; μ_{opt} is the growth rate ($hour^{-1}$) of the species in the food at temperature T_{opt}

Thus the calculation of the potential microbial growth requires four microbial values to be determined: the theoretical growth temperatures (T_{min}, T_{max}, T_{opt}), only on the basis of the studied bacterium; the optimal growth rate (μ_{opt}), depending on the studied bacterium and the type of food.

Calculation of potential *Enterobacter sakazakii* growth

Minimum temperature (T_{min}) was determined at +5.5°C according to Nazarowec-White and Farber (1997a) and optimum (T_{opt}) and maximum (T_{max}) temperatures at +40°C and +46.8°C respectively according to Iversen, Lane and Forsythe (2004).

The growth rates (μ) (or generation times) of *Enterobacter sakazakii* in IMF for different temperatures (Table 1) were estimated using MicroFit® software (Institute of Food Research, Norwich, UK). The optimum growth rate (μ_{opt}) was estimated by fitting the secondary model (Rosso, 1993) using the solver function of Excel. The estimated optimum *Enterobacter sakazakii* growth rate was 2.55 h^{-1}.

According to Nazarowec-White and Farber (1997b), the D-values ranged from 54-79 min. at 52°C and the z value is 5.6°C. These thermotolerance parameters did not allow significant *E. sakazakii* destruction for the collected time temperature profiles, the time over 47°C being relatively short. Thus the model for calculating the potential growth was not modified.

To compare the results, the potential growth results were expressed in decimal log (CFU/g) and 3 groups were defined: below 1 $\log_{10}$; between 1 $\log_{10}$ and 2 $\log_{10}$; above 2 $\log_{10}$.

Comparison with potential *Salmonella* growth

As with any milk product, IMF can be contaminated with *Salmonella*. A recent infant outbreak caused by *Salmonella* Agona in IMF (InVS, 2005; Espié et al., 2005) again highlights the extent to which these potential health risks must be controlled. It was therefore interesting to calculate the potential growth for *Salmonella* as well, and to compare both these health risks.
The infection cases due to *Salmonella* in IMF reported over the last twenty years were caused by different *Salmonella* enterica serovars: Agona (2005) in France (Espié *et al*, 2005; InVS, 2005), London (2000) in Korea (Jong-Ku *et al*, 2004), Anatum (1996) in Europe (Surveillance report, 1997), Bredeney in Australia in 1977 (Forsyth *et al*, 2003), Ealing (1985) in UK (Row *et al*, 1987), Tennessee (1993) in USA and Canada (Centers of Disease Control and Prevention Canada and United States, 1993), Virchow (1994) in Spain (Ruiz *et al*, 1995). Consequently it was decided to determine the growth parameters for *Salmonella* enterica species and not for a defined serovar.

According to Rosso (1995), minimum temperature (T_{min}) was +5°C, optimum (T_{opt}) temperature 40°C and maximum (T_{max}) temperature 47.5°C.
The optimum growth rate (μ_{opt}) was estimated using Growth Predictor® software (Institute of Food Research, Norwich, UK) based on pH values observed in our laboratory for different IMF samples

currently used. The pH average was 6.94 and a_w was the milk value (0,997). The calculated optimum Salmonella *spp* growth rate was 1.76 h^{-1}.

Results and discussion

In 25 neonatal care units, 86 bottle preparations and 93 continuous feeding syringe preparations were studied and 179 time-temperature profiles were collected.

Time-temperature profiles analysis

The time-temperature profiles were analysed in Table 2. They are based on successive stages: preparation in the IMF room, delivery to a neonatal care unit (optional stage), cold storage, bottle reheating, feeding (see Figures 1 and 2).

For the purpose of assessing the impact of the preparation, storage and distribution of IMF on the potential growth results, parameters were defined: initial IMF temperature, time at room temperature, temperature at the end of cold storage, reheating time and temperature at the end of reheating (see Table 2).

Regarding IMF preparation, most of the profiles (74.9%) showed that the IMF was kept at room temperature for a short time (≤ 15 minutes): the bottles/syringes were generally prepared in batches and were then stored in a cold cabinet.

Initial IMF temperature was between 6°C and 18°C in 34% of cases (8 hospitals). It was due to the use of refrigerated water for the IMF preparation in an air-conditioned feeding room. This is quite important as the IMF volume (30 ml) was very sensitive to temperature variations.
In 63.8% of cases (5 hospitals), initial IMF temperature was between 18 and 30°C and was the result of a preparation in a feeding room without any air-conditioning equipment.
Initial IMF temperature of over 30°C was observed in 2.2% of cases (2 hospitals). It was due to water heating, this practice enabled components to mix more easily. In any case, blast chiller was used for quickly cooling down IMF.

Regarding IMF cold storage, the temperature at the end of cold storage depends on the time for which it is stored. The first and last feeding period were therefore analysed separately.
As the first period was short (a few hours after IMF preparation), the profiles (82,8%) with a final cold storage temperature of over 4°C were generally due to a timeframe in the cold cabinet that was too short for cooling down the IMF.
On the other hand, for the last feeding period (around 24 hours after IMF preparation), the profiles (78.2%, 10 care units) with a final cold storage temperature of over 4°C were due to an ineffective or incorrectly regulated cold cabinet. In these care units (10/25 care units) the cold storage cabinet was therefore not able to keep IMF at 4°C or below, according to recommendations (AFSSA, 2005; Tuoby PG. and Jacobs M., 2005; Agostini *et al.*, 2004).

For IMF reheating, microwaves were used in two hospitals (10.5% bottle profiles).
The profiles (36%) with a long reheating time (over 30 minutes) were generally the result of the use of collective hot water batch.

At the end of reheating, the temperature was below 47°C for 54,6% bottles: therefore in case of IMF contamination with *Enterobacter sakazakii* and/or *Salmonella*, microbial multiplication would be possible.

Potential growth analysis

As potential *Enterobacter sakazakii* and *Salmonella spp* growths data were close (see Table 3), potential *Enterobacter sakazakii* growth data were only analyzed.

Bottles (see Table 3):
Most potential *E. sakazakii* growth increments (69%) were under 1 $\log_{10}$ and nearly all (98%) were under 2 $\log_{10}$. In all cases, no significant differences were observed for the feeding period ($p<0,0001$). Only one profile (2%) showed an increment ($\approx 3 \log_{10}$) of over 2 $\log_{10}$. This result was explained by the combination of long storage time (22 hours) and high storage temperature (around +14°C) in an ineffective or incorrectly regulated neonatal care unit cabinet.

Continuous feeding syringes (see Tables 3 and 4):
The potential *E. sakazakii* growth depends mainly on the feeding period (first feeding: 1 hour; last feeding: 3 hours) at room temperature (≥ 25°C). Thus, the maximum increments for this stage were 0,7 $\log_{10}$ for 1 hour feeding time and 2 $\log_{10}$ for 3 hours feeding time.
For one care unit, the situation was completely different: the continuous feeding syringe was placed in a cold cover which kept the IMF temperature under +10°C (+5°C ≤ t ≤ +9.5°C) throughout the feeding period. For 3 hours feeding time, the increment was 0,02 $\log_{10}$.

The 47 profiles (50.5%) with an final increment of under 1 $\log_{10}$ were mainly first feeding profiles (44 profiles). On the other hand the 46 profiles (49.5%) with increment over of 1 $\log_{10}$ were mainly last feeding profiles (41 profiles). Among the increments (7 profiles, 7.5%) of over 2 $\log_{10}$, only one was a first feeding profile. This result could be explained, as with bottles, by the combination of long storage time and high cold storage temperature.

Bottles and continuous feeding syringes (see Table 4):
The phase with the biggest influence on overall potential growth was the cold storage in care units. Fortunately, an ineffective cold storage was exceptional (3 care unit cold cabinets).
Transportation from the IMF room to the care unit, however, (when this stage takes place) had little influence on overall potential growth because this stage was generally short (< 15 min.) or, in the event of longer journeys, the equipment was adapted (cold block in trolley, etc.).

Discussion

The worst potential growth values were explained by a combination of different parameters: high initial IMF temperature (water being warmed before mixing with infant milk formula and being refrigerated without any fast chilling, IMF kept at room temperature for a long time), high storage temperatures (ineffective or incorrectly regulated cold cabinet), long reheating time (collective heating bain marie).

For example, a high initial IMF temperature (27.5°C ≤ t ≤ 33°C for 40 minutes ≈ 0.4 $\log_{10}$) determined no effect on the final potential growth (≈ 1.3 $\log_{10}$) if the cold storage temperature was low and the cold storage time long (+3.5°C≤ t ≤ +7.5°C for 24 hours = 0.01 $\log_{10}$)
On the other hand, air-conditioning equipment in the IMF room could result in an incorrect food safety indication if it was not correctly regulated (air temperature ≈ 20°C) and the IMF was kept at room temperature for a long time (105 minutes, IMF temperature rising from 10 to 20°C ≈ 0.4 $\log_{10}$).

For the continuous feeding syringes, the high potential growth values were mainly due to the feeding period because of the IMF was kept in the infant's room at room temperature (≈ 25°C) during a long time. The other stages have to be also are effective.

The use of a cold cover for keeping the IMF at low temperature in a continuous feeding syringe ensured good food safety, this effect (+5°C ≤ t ≤ +9.5°C during 3 hours), the result (= 0,018 $\log_{10}$) was completely modified by a long storage time in an ineffective or incorrectly regulated cold cabinet (IMF: +5°C ≤ t ≤ +12.5°C for 24 hours ≈ 0.8 $\log_{10}$).

Conclusion

This study assessed temperature conditions in public health establishments for the preparation and storage of infant milk formula (IMF) and feeding it to infants. In order to show the health impact of time-temperature evolution in IMF, *Enterobacter sakazakii* potential growth was calculated.

This assessment showed that the final increment was the result of a combination of different parameters: initial IMF temperature, IMF room temperature, cold storage temperature, cold storage time, reheating temperature, reheating time. One parameter was not usually enough to determine the final increment alone. Each manufacturing stage must be well-controlled and effective, good hygienic practices respected at every stage and the equipment (especially cold cabinets) effective and correctly regulated.

Acknowledgements

The authors are especially grateful to the neonatal care unit managers who participated in this project. The authors would also like to thank Carpentier B., Cornu M. and Poumeyrol G. for their technical help.

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Figure 1

Time-temperature profiles
Bottle profile (first feeding period) and syringe profile (last feeding period)

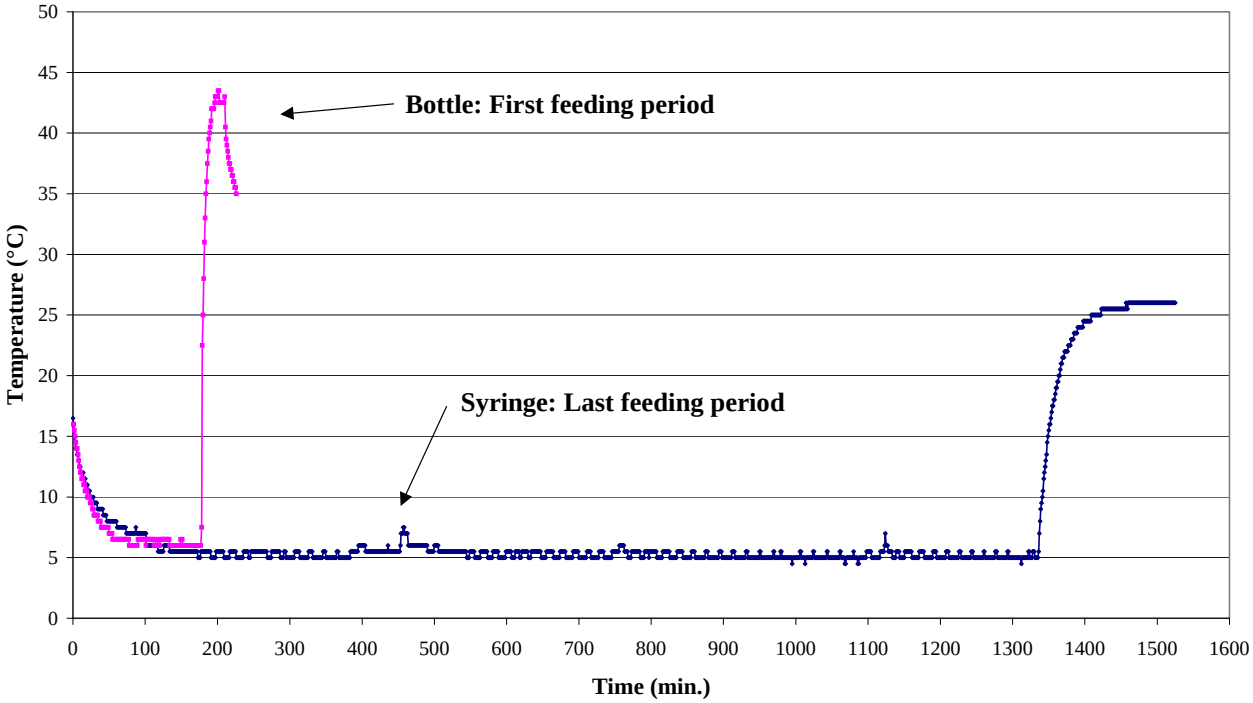

Figure 2
Time-temperature profile: Manufacturing stages (bottle profile)
(a: preparation; b: transport; c: cold storage;
d: reheating; e_1: waiting; e_2: feeding)

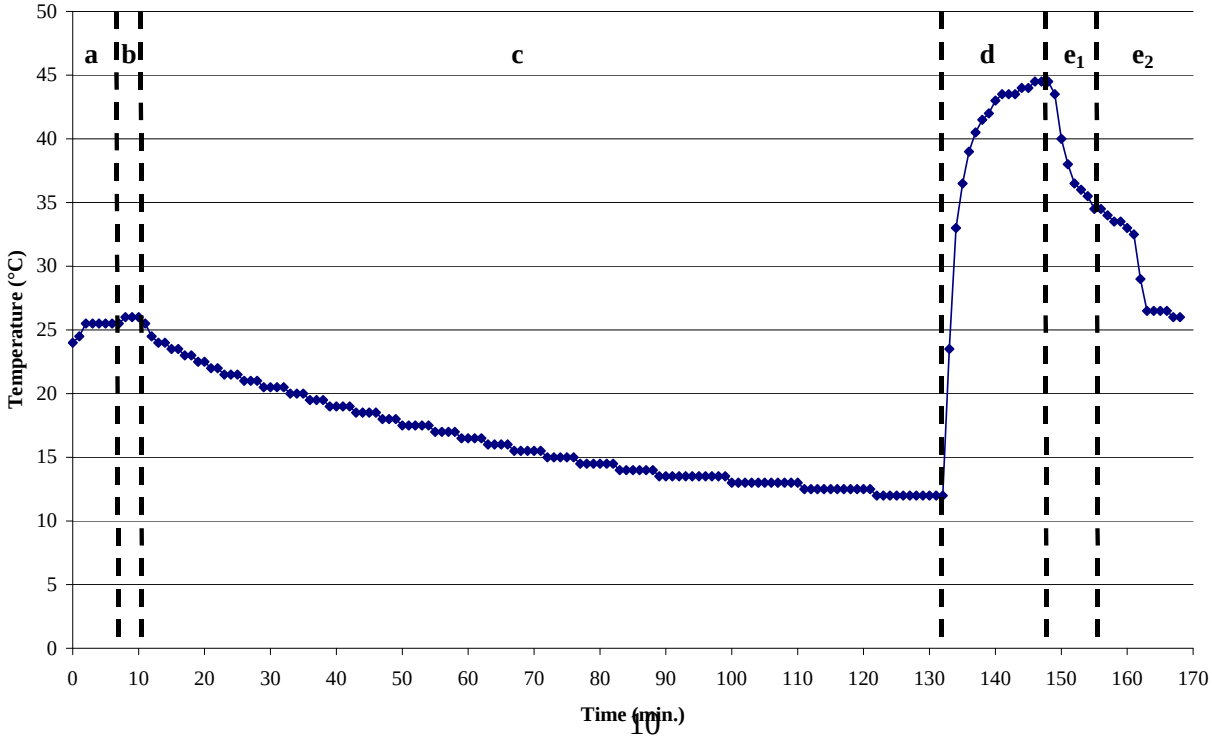

Table 1

Doubling time (h) for *Enterobacter sakazakii* at different temperatures in IMF

Reference	6°C	10°C	21°C	23°C	37°C
Iversen, Lane & Forsythe, 2004	13.7		1.7		19 21
Nazarowec-White & Farber, 1997a		5.52 4.79 5.06 5.12 4.22 4.18 4.20 4.15		0.65 0.85 0.73 0.67 0.66 0.61 0.67 0.66	

Table 2

Analysis of the time-temperature profiles (179)

(86 bottles, 93 syringes)

Parameter	Minimum	Average	Maximum	Repartition of the results		
Time kept at room temperature	1 min.	18 min.	159 min.	*t ≤15 min* 74,9% 134/179	*15 min < t ≤90 min* 19,5% 28/179	*t > 90 min* 5,6% 17/179
Initial IMF temperature (T_0)	6,5°C	20,8°C	36°C	*$T_0 \leq 18°C$* 34% 61/179	*$18°C < T_0 \leq 30°C$* 63,8% 114/179	*$T_0 > 30°C$* 2,2% 4/179
Temperature (T_f) at the end of cold storage	for only first feeding period: -1°C	8,3°C	20°C	*$T_f \leq 4°C$* 17,2% 15/84	*$4°C < T_f \leq 8°C$* 37,9% 33/84	*$T_f > 8°C$* 44,9% 36/84
	for only last feeding period: 0°C	6,2°C	16,5°C	for only last feeding period: 21,8% 19/87	 63,2% 55/87	 15% 13/87
Reheating time duration (t) (for bottles)	25 s.	23 min.	83 min.	*t ≤2 min (microwaves)* 10,5% 9/86	*2 min < t ≤30 min* 53,5% 46/86	*t > 30 min* 36% 31/86
Temperature (T_f) at the end of reheating (for bottles)	31,5°C	46,1°C	62°C	*$T_f \leq 48°C$* 54,6% 47/86	*$48°C \leq T_f \leq 52°C$* 24,4% 21/86	*$T_f > 52°C$* 21% 18/86

Table 3

Potential growth for *Enterobacter sakazakii* and *Salmonella* in IMF

	Growth increment (i) (log_{10} CFU/g)	***Enterobacter sakazakii*** Number of bottles/syringes	***Enterobacter sakazakii*** Percentage (%)	***Salmonella*** Number of bottles/syringes	***Salmonella*** Percentage (%)
Bottles (86)	$i < 1$ (a)	59	69%	76	88%
	$1 \leq i < 2$ (b)	25	29%	9	10%
	$i \geq 2$ (c)	1	2%	1	2%
Feeding syringes (93)	$i < 1$ (a)	47	50,5%	59	63%
	$1 \leq i < 2$ (b)	39	42%	31	33%
	$i \geq 2$ (c)	7	7,5%	3	4%

(a): multiplication factor < 10

(b): multiplication factor ≥ 10 to 100

(c): multiplication factor ≥ 100

Table 4

Potential *E. sakazakii* growth: maximum increments for each stage

	Stage					
	Preparation at room temperature	**Cold storage in IMF room**	**Transport**	**Cold storage in care unit**	**Reheating**	**Feeding**
Bottles: Maximum increment (log_{10}):	1,3	1,1	0,04	2,5	1,1	
Feeding syringes: Maximum increment (log_{10}):	1,2	1,2	0,1	2,9		1 hour: 0,7 3 hours: 2