EFFECT OF INFANT MEAL HOME PREPARATION TEMPERATURE ON SURVIVING OF *BACILLUS CEREUS SENSU LATO*: A CASE OF BECHAR CITY, ALGERIA

– Research paper –

Hassiba DIFALLAH¹, Mohammed ZIANE^{2,3*}, Olfa BEN BRAÏEK⁴, Mohammed BOUAMRA², Habiba LOUZIM⁵

¹*Laboratoire de valorisation des ressources végétales et sécurité alimentaire dans les zones semiaride du Sud Ouest Algérien, Université de Bechar, Algérie*

²*Université de Ain Témouchent, route de Sidi bel abbès, N101, Ain Témouchent 46000, Algérie.*

³*Laboratoire de Microbiologie Appliquée à l'Agroalimentaire, au Biomédical et à l'Environnement (LAMAABE), Faculté des SNV/STU, Université de, Tlemcen 13000 Tlemcen, Algérie*

⁴*Laboratory of Transmissible Diseases and Biologically Active Substances (LR99ES27), Faculty of Pharmacy, University of Monastir, Tunisia*

⁵*MAHU, Laboratoire de pharmacie galénique, Faculté de Médecine de TLEMCEM, Université de Tlemcen*

Abstract: This work aimed to enumerate the *Bacillus cereus sensu lato* from infant's flour sampled at Béchar city and evaluate its resistance to different heating conditions during meal preparation patterns at home. Our findings revealed a prevalence of 74% with 2.4 to 3.9 CFU/g in the analyzed samples. Regarding the heat resistance at 90 °C to 98 °C, our results showed heat resistance variability which depends on the isolate, for example, $D_{90\text{ °C}}$ and $z_{T\text{ °C}}$ values varied from 3.24 to 5.52 min and 11.56 to 89.74 °C respectively. Then, the decimal reduction (n) was calculated at all preparation temperatures (50, 60, 70, 80, 90 and 100 °C). Low "n" was observed with the preparation at $T \leq 50\text{ °C}$ as recommended by the fabricant. However, at the other temperatures, high "n" was observed at 100°C with median and 95th values of 2.22 and 12.36 respectively. Therefore, bacterial concentrations (99th) were estimated at 0.124 log CFU/g for 100 °C. These concentrations could be increased with bacterial growth during meal storage and then achieve critical concentrations. Thus, the results of this work highlight the interest to establish a risk assessment for babies and to improve the production, preparation, and storage conditions of the infant's flour.

Keys words: Inactivation modeling, *Bacillus cereus*, Infant powders, Risk assessment

INTRODUCTION

Since the baby's birth, his feeding is closely linked to breast milk feeding, which provides him an important source of effective infant nutrition for his growth. In addition, it minimizes the risk of allergy, obesity, and intestinal diseases, favors good digestion, and brings antimicrobial factors by supporting infants' immune system (Jackson and Nazar, 2006).

In spite of its importance, breast milk is insufficient to cover the infant needs during the weaning period (Clark and Shrimpton, 2000). Therefore, to ensure the best nutrition for infants, food complementary feeding was introduced as known as the infant's milk formula and the infant's

flour. The infant's milk formula is a substitute of breast milk while the infant's flour could ensure his needs.

In Algeria, the consumption of industrial infant's flour has increased in these last years. Most of the industrial infant's flours commercialized in Algeria are mixtures of several cereals (such as wheat, barley, rye, rice, and corn), mineral complexes, vitamin complexes, sugar, protein and lipids (collected from different labels). This food with low water activity ($a_w < 0.5$) is based on one or several cereal ingredients' ground and dried after being washed to lower their moisture content (NutriFaso, 2007). The wire netting of grains, before fragmentation and dilution in some water or some milk, is made in an oven or a roaster (Sanogo et al., 1994). It is not a sterile product which can contain microorganisms. With its low a_w , bacteria

Received: 28.09.2021

Accepted in revised form: 30.11.2021

* Corresponding author. E-Mail address: ziane.mohammed@yahoo.fr

could not grow, but this fact does not mean the absence of spores forming bacteria because the roasting treatment is not able to eliminate this type of bacteria. *Bacillus cereus* is one of the bacteria that could survive at these conditions. It could be activated and germinate during preparation conditions (hydration and warm water), then could grow during storage to reach a critical concentration (5 log CFU/g). Indeed, at this concentration, *B. cereus* could produce a harmful toxin in the food or in the intestine that is involved in emetic or diarrheal syndromes, respectively (Cadel Six et al., 2012; Savic et al., 2020). Furthermore, it is important to denote that the symptoms caused by these bacteria groups are confused with those caused by *Staphylococcus aureus* and *Clostridium perfringens* (Cadel Six et al., 2012). As a precautionary measure for the baby's preparation, Algerian standards recommend to check *B. cereus* with a maximum limit of 2.7 log CFU/g (JORAD N°39 2017). Otherwise, the manufactures require a temperature preparation between 45-50°C. Unfortunately, at this temperature range, *B. cereus* survives, and thereafter could grow in meal. Ziane et al. (2019) showed that *B. cereus* survives at two and three steaming cycles of couscous semolina at

temperatures ranging from 98 to 100 °C and then grows in the couscous meal. Therefore, different questions could be asked at this level and require further study to obtain accurate answers such as: Does the temperature recommended by fabricant eliminate *B. cereus* in the meal? Do the baby minders follow the fabricant's instructions? Is the baby minder preparation safer than that recommended by the fabricant? Does it necessary to improve a preparation pattern? Thus, this paper aims to assess (1) the preparation patterns of meals based on infant's flour according to different actors (fabricant instructions and baby minders), (2) their heat resistance, (3) the decimal reduction ratio at each preparation temperature, and (4) the concentration of *B. cereus sensu lato* in the infant's flour meal. In this context, it should be important to denote that the *B. cereus sensu lato* is a group of bacteria belonging to *Bacillus* gender and displaying close phylogenetic relationship but are affiliated to different phylogenetic sub-groups (I to VII) with high ecological diversity (Guinebretière et al., 2008). This group includes *B. pseudomycoides*, *B. weihenstephanensis*, *B. mycoides*, *Bacillus anthracis*, *Bacillus cereus sensu stricto*, *Bacillus cytotoxicus* and *Bacillus thuringiensis* (Guinebretière et al., 2008).

MATERIALS AND METHODS

Sampling procedure

To check the infant's flour brands sold in Béchar city, the sampling was conducted at various sale points (grocery stores and pharmacies). Hence, the sampling method was used to spot a random sale point on the map of Béchar city as described by Grawitz (2001). The preparation pattern has been collected from manufacturer's instructions listed on the container of each brand and baby minder behaviors, only for families having a baby. Each preparation pattern defines a scenario.

Microbiological analysis of infant's flour

Detection & enumeration of *B. cereus sensu lato*

Sampling and processing

A total of 70 infant's flour samples with 10 different brands and various tastes were collected from 20 selected sale points, and then transferred to the laboratory in their sold conditions for microbiological analysis. The outside surface of the infant's flour bag was disinfected with alcohol (99.8%) and then opened in aseptic conditions. From each sampling unit, 10 g were taken and diluted in 90 mL of peptone water. The mixture was then put in a water bath at 80°C for 10 min to

eliminate the vegetative flora and select only the spore forming bacteria.

Isolation and enumeration of *B. cereus sensu lato* bacteria

The serial decimal dilutions were made in peptone water from the previously heated mixtures. A volume of 0.1 mL of each dilution was spread over the surface of Mossel Agar plates (Tryptone (10.0 g); Meat extract (1.0 g); D-mannitol (10.0 g); Sodium chloride (10.0 g); Phenol red (25.0 mg); Sterile egg yolk emulsion (100.0 mL); Bacteriological agar (13.5 g); pH 7.2 ± 0.2) (Biokar diagnostics, France), then incubated at 30°C for 24 h for isolation and enumeration. *B. cereus sensu lato* are Gram positive, rod shaped, catalase positive, and endospores forming.

Determination of *B. cereus sensu lato* heat resistance

Production of spore stock

A volume of 0.5 mL from the preculture was spread over the surface of nutrient agar medium (Biokar diagnostics, Beauvais, France) supplemented with 40 mg/L of MnSO₄ and 100 mg/L of CaCl₂, then incubated at 30 °C for the time necessary for the sporulation of the seeded

bacterial population. The sporulation was monitored by observation under the phase contrast microscope for 5 days of incubation. The spores of *B. cereus sensu lato* were then harvested using a sterile spatula by scraping the agar surface. The recovered spores were suspended in 20 mL of sterile distilled water. The prepared spore suspension was centrifuged at $500 \times g$ for 30 min. The pellet was collected and resuspended into 20 mL of sterile distilled water. This operation was repeated twice. The obtained pellet was taken up in a water/ethanol (v/v) mixture and placed at 4°C for 12 h to remove the rest of the vegetative forms. The mixture was then centrifuged at $500 \times g$ for 30 min. The treated pellets underwent three washing cycles with sterile distilled water under the same conditions of centrifugation. The pellets previously recovered were then resuspended in a minimum volume above 200 µL of sterile distilled water to have thus a high concentration of spores. The stock of *B. cereus sensu lato* spores obtained was stored for a minimum one month at 4°C in distilled sterile water before the heat treatment step.

Thermal treatment

A volume of 100 µL of spore stock was suspended in 10 mL of BHI (Brain Heart Infusion Broth) (Biokar diagnostics, Beauvais, France). Then, a volume of 1 mL was distributed in sterile tubes. After heating at different temperature ranged from 90°C to 98°C, the content of each tube was quickly poured into 9 mL of sterile physiological water. Survival spore enumeration was carried out by inclusion of 0.5 mL of heated tubes content into 15 mL nutrient agar, then incubated at 37°C 48 h.

Determination of heat resistance parameters

Heat resistance parameters were estimated by the first order kinetic (Equation 1).

$$\log N_t = \log N_0 - \left(\frac{t}{D_{T^\circ\text{C}}} \right) \quad (1)$$

N_0 represents the initial number of cells, N_t is the number of cells at time t , $D_{T^\circ\text{C}}$ is a time that leads to a 10-fold reduction of surviving population. Otherwise, the influence of temperature ($T^\circ\text{C}$) on bacterial heat sensitivity was quantified by the classical sensitivity parameter $z_{T^\circ\text{C}}$ as shown in Equation 2 (Bigelow, 1921):

$$\log D_{T^\circ\text{C}} = \log D_{T_{\text{mean}}^\circ\text{C}} - \left(\frac{T^\circ\text{C} - T_{\text{mean}}^\circ\text{C}}{z_{T^\circ\text{C}}} \right) \quad (2)$$

$z_{T^\circ\text{C}}$ corresponds to a temperature ($^\circ\text{C}$) increase that leads to a ten-fold reduction of $D_{T^\circ\text{C}}$ -value, $D_{T_{\text{mean}}^\circ\text{C}}$ is a scale parameter (minutes) estimated from the mean of heating temperature $T_{\text{mean}}^\circ\text{C}$ ($^\circ\text{C}$) tested in this study.

Estimation of concentration at the end of each preparation step

Collection of temperature preparation

During meal preparation, lukewarm boiled water (or milk) was added to the infant's flour and then mixed. As a dry product, the infant's flour absorbs quickly the added lukewarm boiled (heating) water (or milk) to achieve the maximum temperature (T_{max}) in the meal, then decreases by cooling.

The preparation way was collected from 100 families. The persons were asked the following questions: (1) Do you respect the meal preparation instructions labeled by the sold brand? (2) Do you know exactly the temperature used for such preparation? (3) If yes, what is it? (4) If no, what is the range of the used temperature: [60-80 $^\circ\text{C}$] or [80-100 $^\circ\text{C}$] ? As for the temperature recommended by the manufacture, it was collected from label instructions (45°C and 50°C).

The temperature profile modeling

To evaluate the temperature profile for all used temperatures (scenarios), the thermometer was emerged in the middle point. To do it, the meal was prepared as follows: a volume of 150 mL of heated water (at the studied temperature for each scenario and collected data) was gradually added to 30 g of infant's flour in the bottle and mixed well to record temperature profile each 1 minute.

Estimation of decimal reduction ratio during preparation

When the temperature profile was recorded, the decimal reduction ratio "n" was estimated at each temperature ($T^\circ\text{C}$) kinetic. The Bigelow method was used, based on calculating the partial biological destruction value ($L(T)$) for each temperature kinetic recorded every minute. Then, the decimal reduction ratio ($n_{T^\circ\text{C}}$) was calculated according to the equation 4. The $L(T)$ were calculated throughout the time spend for the temperature to reach 45 $^\circ\text{C}$. For each onset temperature ($T^\circ\text{C}$) these times were calculated basing on challenge test.

$$L(T) = 10^{\frac{(T^\circ\text{C} - T_{\text{mean}}^\circ\text{C})}{z_{T^\circ\text{C}}}} \quad (3)$$

$$n = \sum L(T) / D_{T_{\text{mean}}^\circ\text{C}} \quad (4)$$

Statistical analysis

The heat resistance parameters ($D_{T^\circ\text{C}}$ and z-values), biological destruction $L(T)$, and decimal reduction ratio (n) were estimated using Excel in Microsoft office. The probabilistic distribution, fitting and Monte Carlo simulation were carried out by @Risk software version 5 (Palisade). As for ANOVA analysis, SPSS software version 22 was used.

RESULTS

Distribution of *B. cereus sensu lato* in analyzed infant's flour samples

The presumptive colonies of *B. cereus sensu lato* are pink and surrounded by an opaque zone due to the mannitol fermentation inability and lecithinase production capacity, respectively.

As for the contamination level in this study, the median concentration ranged between 2.4 to 3.9 log CFU/g

Heat resistance parameters of *B. cereus sensu lato*

To estimate the heat resistance parameters (D_T °C), the linear regression was adopted for each isolate using the whole set of curves. The results are summarized in Table 1. Results show heterogeneity in heat resistance of the studied isolates. The D_T °C values belongs from 3.24 to 5.52, for e.g. 95°C. As for the heat sensitivity, heat treatments were carried out and varied from 11.56°C to 89.74°C.

Estimation of decimal reduction ratio at different preparation temperatures

Meal preparation temperature

As summarized in Table 2, most manufacturers recommend heating the water then letting the temperature drop to 45°C, while no heat treatment is required when using milk for meal preparation. Sixteen percent of the meal preparations used cool or ambient temperature milk and 70% heated it. Others add spices (1.1%) as well as fruits and vegetables (9.8%). The manufacturing does not recommend the use of heated milk may be, to preserve its components and because it underwent a previous sterilization during its production process. According to the objectives of this study, the using frequency is not necessary.

According to a survey conducted in this study, only 41.5% of baby minders respect the recommendations to prepare the infant meal as established by the manufacturers (Table 2), whereas, 49% of them do not respect the label instructions. In fact, these latter baby minders have no information about the safety preparation pattern and they prefer using hot water (more than 60°C) to ensure the death of the likely present microbes (as they called) in the infant's flour without taking into account the guarantee of the nutritional quality of the prepared meals. Effectively, 37.5% and 5.8% of baby minders prepared the infant meal with water heated at 60-80°C and 80-100°C, respectively.

Exposure time during meal preparation

Regarding the exposure time, it depends on the initial temperature. It is the spent time (min) required to achieve the consumption temperature above 45°C. As shown in Table 2, exposure times were calculated for each temperature (50°C, 60°C, 70°C, 80°C, 90°C, 100°C). They were used to calculate the biological destruction value (L(T)).

Temperature profile in the meal preparation

Regardless the tested temperature, the meal temperature decreases. For temperature < 50 °C, the temperature directly achieves the 45°C in 3 min. Moreover, similar observations were noted for scenario 2, when the baby minders (41.7%) respect the manufacturer's instruction. Thus, the same effect could be carried out. As for temperatures 65 and 70°C, they decrease respectively from 54°C and 61°C to 45°C during a recorded time of 10 and 14 minutes respectively.

Estimation of the decimal reduction ratio of *B. cereus sensu lato* in the meal

The biological destruction value (L(T)) was calculated for each temperature kinetic and tested isolate. L(T) was proportional to temperature. The results showed that the high values were observed for 100°C (median=5.40; mean=7.36; 95th=19.92). Then, these values were used to calculate the decimal reduction ratio "n" at each temperature. "n" values were dependent to the preparation temperature and L(T), as reported in Table 2. However, lowest temperature effect was estimated for temperature <50 °C. As shown in Table 2, high values were reported at 100°C (median= 2.22; mean= 3.16; 95th= 12.35), while low ones at 50°C (median= 0.02=mean; 95th= 0.35).

DISCUSSION

It is noted that the prevalence (74%) of *B. cereus sensu lato* seemed to be similar to the prevalence (78%) reported by Heini (2018) and higher than 3.5% reported by Zhang et al. (2017). As for their concentrations in analyzed samples, they were in concordance with those reported (3 to 4 log CFU/g) by Zhang et al. (2017). The presence of *B. cereus sensu lato* in analyzed samples is evident because (1) the infant flour is not a sterile product, (2) and is produced with various ingredients that are generally contaminated by *B. cereus* group. In fact, several infant's flour ingredients are contaminated by *B. cereus sensu lato* mainly rice (Sarrias et al. 2002), maize (Byaruhanga et al. 1999), wheat (Valerio et al. 2012) or oats and other cereals (Daczowska-Kozon et al. 2009). The *B.*

cereus sensu lato group is mostly present in raw materials, which increases its probability and/or confirms its occurrence in the infant's flour.

The presence of *B. cereus sensu lato* in the infant's flour could be due to several factors: (1) the contaminated raw material, (2) the ability of *B. cereus sensu lato* to produce a spore form to survive in hostile conditions, especially at low a_w of this product (infant's flour). Fortunately, at this step, the *B. cereus sensu lato* is not exceeding a critical concentration (5 log CFU/g) and could decrease after preparation with warm boiling water depending to the preparation patterns.

The problem is not related only to the *B. cereus sensu lato* existence in the product, but also to (1) its heat resistance and ability to grow and achieve a critical concentration (5 log cells per g) and (2) low temperature of preparation for both patterns (manufacturing instructions and baby minder preparation). Thus, the *B. cereus* level could increase particularly when they survive and growth during preparation and storage respectively. In fact, Sadek et al. (2018) considered infant's formula as an excellent medium for a bacterial growth. Buss da Silva et al. (2017) showed the growth of three tested strains of *B. cereus* in reconstituted infant formula. Otherwise, Messelhäuser et al. (2014) reported that cereal-based infant food formula, rich in vitamins and trace elements, is known to promote cereulide synthesis.

According to the rarity of studies in this field, it could be given as example, the couscous semolina as similar product (low a_w and heating preparation). In this context, Ziane et al. (2019) reported the growth of *B. cereus* in couscous semolina after streaming at $>90^\circ\text{C}$ for two or three cycles during above 15 min (a heating condition more severe than that using for infant flour meal preparation). These results depended on the storage and waiting time. Thus, the waiting time to finish a meal is important to prevent against obtaining unsafe meal or exposing baby to critical concentration of toxigenic *B. cereus*.

As for the heat resistance of tested strains of *B. cereus sensu lato*, the $D_{95\text{ }^\circ\text{C}}$ values are in disagreement with those (4.4-8.4 min and 0.68-18.85 min) reported by Stoeckel et al. (2013) and Ziane et al. (2014) respectively. According to our findings, the heat resistance is strain dependent, similar to the results exposed by Ziane et al. (2014) and Ziane et al. (2016). Otherwise, the $z_T\text{ }^\circ\text{C}$ values were higher than those reported by Mazas et al. (1999), Janštová and Lukášová (2001), and Montville et al. (2005), which ranged from $7\text{ }^\circ\text{C}$ to $13\text{ }^\circ\text{C}$.

The previously heat resistance parameters were

used to estimate the decimal reduction number "n" at different scenario. As a matter of fact, the low "median of 0.02" was reported for the low temperature preparation recommended by fabricant.

Unfortunately, temperature recommended by fabricant is insufficient to reduce significant the *B. cereus* concentration (Table 3).

The *B. cereus sensu lato* concentration at different preparations was estimated by Equation 5 developed by Nauta (2001) and Membré and Valdramidis (2016). The probabilistic approach was used to simulate the *B. cereus sensu lato* concentration using Poisson distribution:

$$[B. cereus sl]_s = \text{Riskpoiss}([B. cereus sl]_0 \times m_0 \times 10^{-n}) \quad (5)$$

where

$[B. cereus sl]_s$: survival concentration (CFU per prepared amount) of *B. cereus sensu lato* in the meal after heating;

$[B. cereus sl]_0$: Distribution of the initial concentration (CFU per gram of analyzed infant's flour) of *B. cereus sensu lato*. It was fitted by RiskDuniform ($C_1, C_2, C_3, \dots, C_i$) $\times$ RiskBeta (number of contaminated samples + 1, no contaminated samples+1). C_1 to C_i are the concentrations of *B. cereus sensu lato* in each positive sample (1 to i respectively). As for the probability of toxigenic strains occurred among these concentrations, the RiskUniform (0;1) distribution was used. The results were reported in Table 2. In the absence of information about distribution of toxigenic bacteria among *B. cereus* group, it was assumed that toxigenic *B. cereus* could represent any percentage (varying from 0 to 100%) of all group of *B. cereus* that was therefore described by a RiskUniform(0;1) as shown by Ziane et al. (2019).

n: corresponds to the decimal reduction ratio, that was estimated for each isolate at each temperature. The "n" values were derived from Equation 5 of RiskDuniform distribution (RiskDuniform($n_1, n_2, \dots, n_{12}$)), with the index of "n" representing the isolate number that varied from 1 to 12.

m_0 : quantity (g) of infant flour in package (250g). Because the isolates were not affiliated in their toxigenic groups to have information about their pathogenicity, thus, they were assumed to exhibit toxigenic groups with equiprobability. It was implemented in the estimation of RiskUniform (0;1).

Table 1. Heat resistance parameters D values (min) and z_T values ($^{\circ}\text{C}$) of survival *B. cereus sensu lato* at different temperatures ranged between 90 and 98 $^{\circ}\text{C}$. R is the correlation coefficient. Confidence intervals at 95% are given in brackets. Confidence intervals of z-values are not significant.

	<i>B. cereus</i> IF1	<i>B. cereus</i> IF2	<i>B. cereus</i> IF3	<i>B. cereus</i> IF 4	<i>B. cereus</i> IF 5	<i>B. cereus</i> IF 6	<i>B. cereus</i> IF 7	<i>B. cereus</i> IF 8	<i>B. cereus</i> IF 9	<i>B. cereus</i> IF 10	<i>B. cereus</i> IF 11	<i>B. cereus</i> IF 12
$D_{90}^{\circ\text{C}}$ (min)	3.24 [3 - 3.48] $R^2 = 0.981$	4.95 [4.49 - 5.41] $R^2 = 0.983$	4.95 [4.12 - 5.78] $R^2 = 0.947$	5.52 [4.75 - 6.29] $R^2 = 0.962$	4.9 [4.37 - 5.43] $R^2 = 0.977$	3.87 [3.49 - 4.25] $R^2 = 0.987$	4.45 [4.38 - 4.52] $R^2 = 0.999$	5.15 [4.19 - 6.11] $R^2 = 0.985$	3.72 [3.23 - 4.21] $R^2 = 0.967$	5.25 [4.67 - 5.83] $R^2 = 0.938$	5.24 [4.6 - 5.88] $R^2 = 0.971$	5.4 [4.44 - 6.36] $R^2 = 0.94$
$D_{95}^{\circ\text{C}}$ (min)	2.96 [2.91 - 3.01] $R^2 = 0.993$	3.05 [2.77 - 3.33] $R^2 = 0.983$	3.59 [3.34 - 3.84] $R^2 = 0.99$	3.21 [3.13 - 3.29] $R^2 = 0.999$	3.16 [2.85-3.47] $R^2 = 0.982$	2.77 [2.67-2.87] $R^2 = 0.997$	3.05 [2.84-3.26] $R^2 = 0.99$	3.08 [2.96-3.2] $R^2 = 0.997$	1.52 [1.51-1.53] $R^2 = 0.91$	3.17 [2.67-3.67] $R^2 = 0.982$	4.62 [3.56-5.68] $R^2 = 0.905$	1.76 [1.51-2.01] $R^2 = 0.987$
$D_{98}^{\circ\text{C}}$ (min)	1.38 [1.23 - 1.53] $R^2 = 0.989$	1.99 [1.8 - 2.19] $R^2 = 0.9$	2.97 [2.59 - 3.35] $R^2 = 0.969$	2.12 [1.93 - 2.31] $R^2 = 0.992$	2.87 [2.53 - 3.21] $R^2 = 0.972$	1.76 [1.26 - 2.26] $R^2 = 0.986$	2.85 [2.75-2.95] $R^2 = 0.998$	2.77 [2.46 - 3.08] $R^2 = 0.997$	1.02 [0.79 - 1.25] $R^2 = 0.987$	2.16 [2.01 - 2.31] $R^2 = 0.991$	4.26 [4.17 - 4.37] $R^2 = 0.972$	1.12 [0.96 - 1.28] $R^2 = 0.987$
z_T -value ($^{\circ}\text{C}$)	21.80 $R^2 = 0.9954$	20.58 $R^2 = 0.9889$	36.04 $R^2 = 1$	19.43 $R^2 = 0.9956$	33.37 $R^2 = 0.9563$	24.17 $R^2 = 0.9478$	39.89 $R^2 = 0.9442$	28.75 $R^2 = 0.9525$	14.11 $R^2 = 0.9942$	20.96 $R^2 = 0.9959$	89.74 $R^2 = 0.9998$	11.56 $R^2 = 0.9904$

Table 2. Estimation of biological destruction value $L(T)$, decimal reduction number (n).

	%	Temperature T ($^{\circ}\text{C}$)	Maximum temperature in meal	Time need to achieve temperature consumption 45 $^{\circ}\text{C}$	$L(T)$			Decimal reduction number "n"		
					mean	median	95 th	mean	median	95 th
Label instruction	//	≤ 50	47	4	0.24	0.06	1.62	0.02	0.02	0.35
Baby minder	41.7	≤ 50								
	5.8	60-80	60	10	0.62	0.18	3.72	0.18	0.10	0.79
			70	14	1.08	0.40	5.79	0.33	0.20	1.23
	37.5	80-100	80	17	1.99	0.95	9.12	0.67	0.42	1.94
			90	30	4.80	3.06	16.71	1.83	1.23	6.16
			100	4	7.36	5.40	19.92	3.16	2.22	12.36

Figure 1. Evolution of *B. cereus sensu lato* (log CFU/g) during preparation.

Table 3. Estimation *B. cereus sensu lato* concentration (log CFU/g of meal) at different preparation temperatures of infant's flour meal.

T °C	CFU/g				ANOVA analysis			
	Before preparation		preparation					
	min	median	mean	99 th	min	mean	median	99 th
=<50	1.729	2.489	2.615	3.705	1.158	1.06	3.11	0.001
60					0.939	0.828	2.908	
70					0.746	0.584	2.756	
80					0.4878	0.304	2.36	
90					0.142	0.036	1.126	
100					0.0135	0.004	0.124	

The Monte Carlo simulations of initial concentrations of toxigenic *B. cereus* in the infant's flour before preparation were reported in Table 3. It was assumed at (median = 2.489; mean = 2.615; 99th = 3.75) log CFU/g.

After preparation, the eventual toxigenic *B. cereus* concentrations were reported in Table 3 according to Equation 5. The low values (CFU per g of prepared meal) were estimated for temperature 100

°C (min=0, median = 0.004; mean = 0.135; 99th = 1.24 log CFU/g) followed by scenario 90 °C (min=0, median = 0.036; mean = 0.142, 99th = 1.226 log CFU/g) (Table 3). Both of these temperatures are safe for infants, while heating at temperature recommended by the manufacturers is hazardous and no significant effect (ANOVA $P<0.001$) change in initial concentration was observed (Figure 1).

CONCLUSION

Nowadays, the use of infant's flour by parents has known a significant increase. However, in spite of its weak water activity, this food product is non-sterile and several undesirable and pathogenic microorganisms could proliferate there. Therefore, great attention should be paid to the consumption of infant's flour in terms of the occurrence of potential microbial dangers such as spores since it is intended for a sensitive group of consumers (infants).

In this line, this study was conducted to search these bacteria in such food stuff, especially the *B. cereus sensu lato* that are known for their heat resistance. According to the preparation patterns, *B. cereus sensu lato* could be involved in food poisoning.

Our findings showed that 75% of infant's flour samples were contaminated with *Bacillus*. In addition, the results of this paper highlight the effectiveness in reducing the level of *B. cereus sensu lato* compared to the manufacturer's instructions. Thus, until it is difficult to increase a

heating temperature, that could reduce the nutritional quality of the infant meal, only the patterns use must be improved. Thus, some recommendations could be established to avoid issues related to this type of bacteria:

- Choose a relevant raw material;
- Improve continuously the process and control it;
- Review the preparation instructions and improve the product solubility at high temperatures;
- Reduce the amount of flour from 200 g to 100 g per package;
- Add the storage condition to the packaging.

Finally, as perspectives for this study, additional experiments need to be performed in the future, on:

- (1) determining the *B. cereus* affiliation into different groups;

- (2) studying their growth in the infant's flour meal;
- (3) conducting the microbial risk assessment to get in-depth sufficient details about this type of bacteria which will undoubtedly help industries during production process and stores during storage.

REFERENCES

1. Bigelow, W. D. (1921). The logarithmic nature of thermal death time curves. *J. Infect. Dis.* 29, 528-536. <https://www.jstor.org/stable/30082910>
2. Buss da Silva, N., Baranyi, J., Carciofi, B. A. M. & Ellouze, M. (2017). From culture-medium-based models to applications to food: predicting the growth of *B. cereus* in reconstituted infant formulae. *Front. Microbiol.* 8, 1799. doi: 10.3389/fmicb.2017.01799
3. Byaruhanga, Y. B., Bester B. H. & Watson T.G. (1999). Growth and survival of *Bacillus cereus* in mageu, a sour maize beverage. *World J. Microbiol. Biotechnol.* 15, 329-333. <https://doi.org/10.1023/A:1008967117381>
4. Carlin, F, Albagnac, C, Rida, A, Guinebretière, M. H, Couvert O. & Nguyen-The C. (2013). Variation of cardinal growth parameters and growth limits according to phylogenetic affiliation in the *Bacillus cereus* Group. Consequences for risk assessment. *Food Microbiol.* 33, 69-76. DOI: [10.1016/j.fm.2012.08.014](https://doi.org/10.1016/j.fm.2012.08.014)
5. Clark, D. & Shrimpton R. (2000). Complementary feeding, the Code, and the Codex. *Food Nutr. Bull.* 21, 25-29.
6. Dackowska-Kozon, E. G., Bednarczyk, A., Biba M. & Repich K. (2009). Bacteria of *Bacillus cereus* group in cereals at retail. *Polish J. Food Nutr. Sci.* 59, 53-59.
7. Guinebretière, M. H., Thompson, F. L., Sorokin, A., Normand, P., Dawyndt, P., Ehling-Schulz, Svensson, B., Sanchis, V., Nguyen-The, C., Heyndrickx, M. & De Vos, P. 2008. « Ecological diversification in the *Bacillus cereus* group ». *Environ. Microbiol.* 10, 851-865. <https://doi.org/10.1111/j.1462-2920.2007.01495.x>
8. Grawitz M. (2001). Méthodes des sciences sociales (Social science methods). 11^{ème} édition. 2001. Paris: Dalloz.
9. Heini, N. M., Stephan, R., Stephan R. & Ohler S. (2018). Characterization of *Bacillus cereus* group

- isolates from powdered food products. *Int. J. Food Microbiol.* 283, 59-64. <https://doi.org/10.1016/j.ijfoodmicro.2018.06.019>
10. Jackson, K. M. & Nazar AM. (2006). Breastfeeding, the Immune Response, and Long-term Health. *J. Am. Osteopath. Assoc.* 106, 203-207. <https://pubmed.ncbi.nlm.nih.gov/16627775/>
 11. Janštová, B. & Lukášová J. (2001). Heat resistance of *Bacillus* spp. spores isolated from cow's milk and farm environment. *Acta Vet Brno* 70, 179-184. DOI: 10.2754/avb200170020179
 12. Mazas, M., López, M., Martínez, S., Bernardo A. & Martin R. (1999). Heat resistance of *Bacillus cereus* spores: effects of milk constituents and stabilizing additives. *J. Food Prot.* 62, 410-413. DOI: 10.4315/0362-028x-62.4.410
 13. Membré, J. M. & Valdramidis V. (2016). Modeling in Food Microbiology. From Predictive Microbiology to Exposure Assessment. ISTE Press Ltd and Elsevier Ltd, UK. <https://doi.org/10.1016/B978-1-78548-155-0.50005-8>
 14. Messelhäuser, U., Frenzel, E., Blöching, C., Zucker, R., Kämpf, P. & Ehling-Schulz, M. (2014). Emetic *Bacillus cereus* are more volatile than thought: recent foodborne outbreaks and prevalence studies in Bavaria (2007-2013). *Biomed Res. Int.* 2014:465603
 15. Montville TJ, Dengrove R, De Siano T, Bonnet M, & Schaffner DW. (2005). Thermal resistance of spores from virulent strains of *Bacillus anthracis* and potential surrogates. *J. Food Prot.* 68, 2362-2366. DOI: 10.4315/0362-028x-68.11.2362
 16. Nauta, M. J. (2001). A modular process risk model structure for quantitative microbiological risk assessment and its application in an exposure assessment of *Bacillus cereus* in a REPFED. RIVM Report 249106007. Bilthoven, The Netherlands. <http://hdl.handle.net/10029/9385>
 17. NutriFaso, (2007). Cahier des charges d'une farine infantile souhaitant être labellisée NUTRIFASO (Specifications for infant flour wishing to be labeled). Projet NutriFaso, Gret, IRD, ANSA-B.
 18. Sadek, Z.I., Abdel-Rahman, M.A., Azab, M.S., Darwesh, O.M., Hassan, M.S. (2018). Microbiological evaluation of infant foods quality and molecular detection of *Bacillus cereus* toxins relating genes. *Toxicol. Rep.* 5, 871-877. <https://doi.org/10.1016/j.toxrep.2018.08.013>.
 19. Sanogo, M., Branderhorst, E., Laurent F. & Trèche S. (1994). La production artisanale des farines infantiles, Expériences et procédés (The artisanal production of infant flours ériences Experiences and processes). Gret, Paris, France; 11.
 20. Sarrias, J. A., Valero M. & Salmeron MC. (2002). Enumeration, isolation and characterization of *Bacillus cereus* strains from Spanish raw rice. *Food Microbiol.* 19, 589-595. <https://doi.org/10.1006/fmic.2002.0514>
 21. Savić, D., Ristanović, E., Miljković, S. B., Radaković, S., Jošić, D. & Lepšanović, Z., (2020). Enterotoxin and emetic toxin genes profiles and genetic diversity of *Bacillus cereus* isolated from food, environmental and clinical samples in Serbia. *Acta Veterinaria*, 70, 182-193. DOI: <https://doi.org/10.2478/acve-2020-0013>
 22. Stoeckel, M., Westermann, A. C., Atamer Z. & Hinrichs J. (2013). Thermal inactivation of *Bacillus cereus* spores in infant formula under shear conditions. *Dairy Sci. Technol.* 93, 163-175. <https://hal.archives-ouvertes.fr/hal-01201403>
 23. Valerio, F., De Bellis, P., Di Biase, M., Lonigro, S.L., Giussani, B., Visconti, A., Lavermicocca P. & Sisto A. (2012). Diversity of spore-forming bacteria and identification of *Bacillus amyloliquefaciens* as a species frequently associated with the ropy spoilage of bread. *Int. J. Food Microbiol.* 156, 278-285. <https://doi.org/10.1016/j.ijfoodmicro.2012.04.005>
 24. Zhang, Y., Chen, J., Feng, C., Zhan, L., Zhang, J., Li, Y., Yang, Y., Chen, H., Zhang, Z., Zhang, Y., Mei L. & Li H. (2017). Quantitative prevalence, phenotypic and genotypic characteristics of *Bacillus cereus* isolated from retail infant foods in China. *Foodborne Pathog. Dis.* 14, 564-572. DOI: [10.1089/fpd.2017.2287](https://doi.org/10.1089/fpd.2017.2287)
 25. Ziane, M., Couvert, O., Le Chevalier, P., Moussa-Boudjemaa B. & Leguerinel I. (2016). Identification and characterization of aerobic spore forming bacteria isolated from commercial camel's milk in south of Algeria. *Small Ruminant Res.* 137, 59-64. <https://doi.org/10.1016/j.smallrumres.2016.03.004>

26. Ziane, M., Desriac, N., Le Chevalier, P., Moussa-Boudjemaa B. & Leguerinel I. (2014). Identification, heat resistance and growth potential of mesophilic spore-forming bacteria isolated from Algerian retail packaged couscous. *Food Control* 45, 16-21. <https://doi.org/10.1016/j.foodcont.2014.04.003>
27. Ziane, M., Leguerinel I. & Membré J.M. (2019). A quantitative microbiological exposure assessment of *Bacillus cereus* group IV in couscous semolina, Algeria. *Microb. Risk Anal.* 11, 11-22. <https://doi.org/10.1016/j.mran.2018.07.001>