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Distribution of Cronobacter spp. in industrial batches of powdered infant formula and the impact of sampling approaches

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Abstract

The spatial distribution of pathogenic microorganisms within a batch of food will influence the results of sampling for microbiological testing and will also influence the public health risk. However, knowledge about how microorganisms are actually spatially distributed in foods is scarce. This study investigates how *Cronobacter* spp. are distributed on batch-scale throughout a recalled batch of powdered infant formula (PIF) and it investigates on local-scale the occurrence of clusters of *Cronobacter* cells. Additionally, the performance of typical sampling plans and strategies are investigated. The concentration of *Cronobacter* spp. was assessed in the course of the filling time by taking samples of 333 g using the most probable number (MPN) enrichment technique. Since estimating concentrations by enrichment does not distinguish between a single cell or clusters of cells, the occurrence of clusters of *Cronobacter* spp. cells was investigated by plate counting 2290 samples of 1 g. In the recalled batch 415 MPN samples were drawn and in 58% the concentrations were estimated to be below the detection limit of -2.52 log CFU/g. *Cronobacter* spp. were heterogeneously distributed throughout the batch with parts with no detectable contamination and parts with concentrations between -2.52 and 2.75 log CFU/g. Clusters of cells occurred sporadically in 8 out of 2290 samples. The two largest clusters contained 123 (2.10 log CFU/g) and 560 (2.75 log CFU/g) cells. The concentration in the reference batch was -4.4 log CFU/g, 99% of the 93 samples were below the detection limit. Various sampling plans were evaluated for the contamination data from the recalled batch. Taking more and smaller samples and keeping the total sampling weight constant, improved the performance of the sampling plans to detect such a type of contaminated batch.

Keywords: *recalled batch, heterogeneity, probability, sampling plan, lot*

Introduction

There is little known about how microorganisms are actually spatially distributed in foods. In many cases, generalising or default assumptions are made regarding the spatial distribution and appropriate sampling strategies. According to Kilsby and Baird-Parker (1983), the total viable counts from batches including frozen meat, frozen vegetable, and frozen dairy products appeared to be lognormally distributed in 92% of the batches; in 8% of the batches the total viable count appeared to be not lognormally, with a maximum of 13% for powdered products. Based on studies including the findings of Kilsby and Baird-Parker, the International Commission on Microbiological Specification for Foods (ICMSF 2002) assumed a Lognormal distribution in order to evaluate the performance of attribute sampling plans. According to the ICMSF (2002), a standard deviation of 0.8 log CFU/g was chosen based on data derived from the meat industry (Greenberg *et al.* 1966) and similar observations in other food products. Assuming a lognormally distributed contamination, also the size of the standard deviation will affect the performance of a sampling plan (Legan et al. 2001). Habraken et al. (1986) established that substantial clustering of contamination occurs in dried milk products, with parts of the batch containing microorganisms and other parts containing no microorganisms at all. This clustering or heterogeneity will make the interpretation of the sampling results difficult. Besides heterogeneity on batch-scale, heterogeneity on local-scale is possible within the food product. One could speculate that bacteria may grow overnight to levels of 10^9 cells/mL in a droplet of water and powder. This may result in clusters of cells

with high concentrations, which may influence both risk assessments and public health. In order to investigate the spatial distribution of microorganisms in a batch of food and its impact on various sampling approaches, powdered infant formula (PIF) was chosen as product and *Cronobacter* spp. as target microorganism.

Powdered infant formulae (PIF) given to infants during the first months of life needs to be manufactured according to very stringent hygiene measures, since PIF has been linked to outbreaks related to the presence of *Cronobacter* spp. (FAO/WHO 2006; CAC 2008; Cordier 2008). Currently every batch of PIF has to be tested for *Cronobacter* spp. by drawing 30 samples of 10 g according direct sampling plans (CEC 2007). In a recent FAO/WHO risk assessment (FAO/WHO 2006) the mean concentration and standard deviation of *Cronobacter* spp. in batches of powdered infant formula have been estimated from prevalence data to be respectively, -3.8 log CFU/g and 0.7 log CFU/g.

This study investigated the distribution of *Cronobacter* spp. within a batch of PIF, that had been recalled after *Cronobacter* spp. had been detected. For comparison a reference batch produced in the same factory was investigated in detail as well. Estimating low microbial concentrations with the most probable number (MPN) technique by enrichment does not distinguish between a single cell or clusters of cells. Therefore, additionally on local-scale the occurrence of clusters of *Cronobacter* spp. cells was investigated by plate counting many small samples. Thereafter, the performances of various sampling plans were calculated.

Materials and Methods

Investigating batches of powdered infant formula

To assess the distribution of *Cronobacter* spp. in batches PIF, 415 samples of 333 g from the recalled batch and 93 samples from the reference batch were investigated. The concentration of *Cronobacter* spp. was estimated in samples of 333 g using the Most Probable Number (MPN) technique (3 x 100 g, 3 x 10 g , and 3 x 1 g) and the screening method as published by Iversen et al. (2008). To investigate the presence of local clusters of cells, 28 bags were chosen with high concentrations or concentrations below the detection limit of -2.52 log CFU/g (0.003 CFU/g). The remaining powder was divided in samples of 1 gram and all samples were diluted in 9 ml of PPS and 3 ml of the suspension was pour plated in Trypton Soy Agar with sodium pyruvate at a concentration of 0.1 % (wt/vol) (TSAP) and a top layer of TSAP. Sodium pyruvate was added in order to enhance the resuscitation of stressed *Cronobacter* spp. cells during plating (Gurtler and Beuchat 2005). Since 3 ml of the -1 dilution was plated, the lower detection limit was 3.3 CFU/g for a sample size of 1 g,

Random sampling

By randomly drawing a number of samples (*n*) with a specific sample size from the data set, the probability that the sampling scheme includes one or more positive samples $\Pr(n_+ > 0)$ can be calculated as follows:

$$\Pr_{rand}(n_+ > 0) = 1 - (1 - s_+)^n \qquad (1)$$

with: *n*: number of samples; n_+: number of positive samples; s_+: fraction of positive samples of a specific sample size. Since the data set contained information on triplicate samples of 100, 10, and 1 g, it was also possible to assess fractions of positive samples for sample sizes of 300, 30, and 3 g.

Results and Discussion

Distribution of Cronobacter *spp. in PIF*

On batch-scale, the distribution of *Cronobacter* spp. cells throughout a recalled and a reference batch was investigated by relating concentrations to the time that the bag is actually filled. Nearly 60 % of the MPNs had an MPN code of 0,0,0 and the concentration was estimated below the detection limit of -2.52 log CFU/g (0.003 CFU/g). Two samples had a concentration estimated above the upper detection limit of 0.041 log CFU/g (1.1 CFU/g) for

an MPN of 333 g. Figure 1 shows the empirical cumulative distribution function (ECDF) of the samples drawn from the recalled and reference batch.

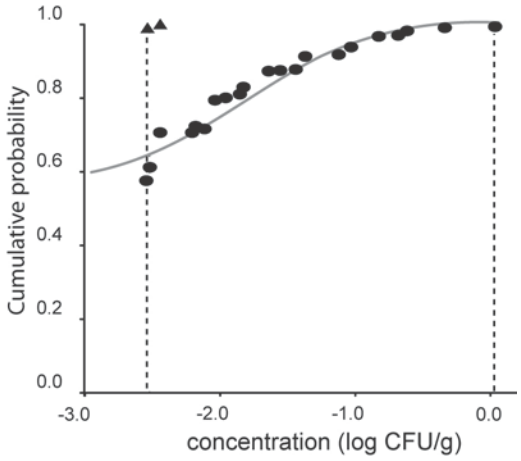

Figure 1 Empirical cumulative distribution functions of the concentrations of *Cronobacter* spp. (log CFU/g) in MPNs of 333 g drawn from the (▲) reference and (●) recalled batch. The grey curve represents a Normal distribution with a mean -1.779 log CFU/g and standard deviation 0.675 log CFU/g of the positive samples (y = 0.42 x Normal (-1.779, 0.675) + 0.58). The dotted vertical lines indicate the lower (-2.52 log CFU/g) and the upper (0.041 log CFU/g) detection limits.

On local-scale 2272 samples of 1 g were below the detection limit of 3.3CFU/g and 8 samples varied between 3.3 and 560 CFU/g and two concentrations peaked at 123.3 and 560 CFU/g.

The probability that the sampling scheme includes one or more positive samples by random ($\Pr_{rand}(n_+ > 0)$*) sampling*

Table 1 shows $\Pr_{rand}(n_+ > 0)$, the probability that the sampling scheme includes one or more positive samples, by drawing random samples from the recalled and reference batch. Eq. 1 and the fractions of positive samples were used to calculate $\Pr_{rand}(n_+ > 0)$. Table 1 shows that keeping the total sample weight constant at 300 g and increasing the number of samples from 1 to 30, increases $\Pr_{rand}(n_+ > 0)$ from 0.378 till 0.999.

Table 1: The probability ($\Pr_{rand}(n_+ > 0)$) that the entire sampling scheme contains one or more positive samples by sampling randomly with various numbers of samples and sample sizes from the recalled and the reference batch. $\Pr_{rand}(n_+ > 0)$ was calculated with Eq. 1

Total sample weight (g)	Number of samples	Sample size (g)	Recalled batch $\Pr(n_+ > 0)$	Reference batch $\Pr(n_+ > 0)$
300	1	300	0.378	0.0118
300	3	100	0.612	0.011
300	10	30	0.896	-[a]
300	30	10	0.969	-[a]
300	100	3	0.999	-[a]

[a] No positive sample available with this sample size

Conclusions

Thorough investigation of the recalled batch showed that *Cronobacter* spp. were heterogeneously distributed throughout the batch containing parts with no detectable contamination and parts with concentrations varying between -2.52 and 2.75 log CFU/g. Clusters of cells occurred sporadically in 8 out of 2290 samples of 1 g. The two largest clusters contained 123 (2.10 log CFU/g) and 560 (2.75 log CFU/g) cells. Taking more and smaller samples and keeping the total sampling weight constant, improved the performance of the sampling plans to detect such a type of contaminated batch.

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