Wet heat treatment of Cronobacter sakazakii and detection of viable cells using RT-PCR and Propidium monoazide for distinction between dead and viable cells

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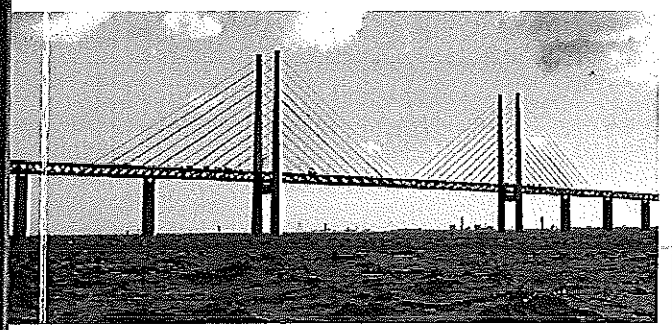

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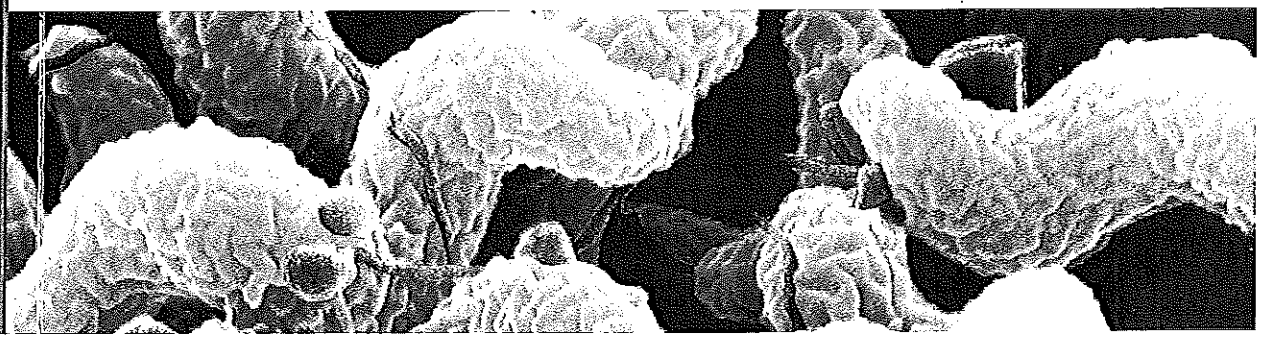

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PED2.50 Wet heat treatment of *Cronobacter sakazakii* and detection of viable cells using RT-PCR and Propidium *monoazide* for distinction between dead and viable cells

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The food borne pathogen *Cronobacter sakazakii*, previous known as *Enterobacter sakazakii*, is associated with powdered infant formulas (PIF). Because *C. sakazakii* is related to outbreaks of meningitis, septicaemia and necrotizing enterocolitis in neonates it is of great interest to obtain a better understanding of the heat treatment which is needed to inactivate *C. sakazakii*. We propose a new way of evaluating heat treatment by use of Propidium Monoazide RT-PCR. Propidium Monoazide (PMA) enters the nucleus of bacterial cells with damaged membranes (non-viable). Upon light exposure PMA is converted into a highly unstable nitrine intermediate with a high affinity for DNA. The nitrine intermediate is quickly covalently bound to the DNA present. After light exposure DNA is extracted from viable and non-viable cells using commercially available DNA extraction kits. PMA bound DNA will thus be extracted together with the non-PMA bound DNA. DNA, which is bound to PMA, is no longer capable of interacting in PCR reactions and it is therefore possible to use quantitative RT-PCR to determine the number of viable cells. In the present study a primer set specific *C. sakazakii* was used in the RT-PCR. The PMA RT-PCR method was used to determine D-values and a z-value for *C. sakazakii* cells after heat treatment. For verification purposes CFU data was used. Thermal inactivation of *C. sakazakii* DSM 4485^T was conducted in triplicates, D-values and a z-value was obtained. Heat treatment was carried out in Erlenmeyer flasks containing BHI-broth preheated to either 52 °C, 54 °C 58 °C and at 60 °C. The corresponding D-values were found to be 46.5 min, 10.56 min, 3 min and 1.6 min as obtained from CFU determinations. For the PMA RT-PCR determination of viable cells 1 mL of heat treated *C. sakazakii* cells was centrifuged (14.000g) for 5 min at 5 °C and the supernatant was discarded. The pellet was suspended in 0.55 mM PMA for 10 min in the dark at 5 °C, with 5 sec vortexing every 2 min. The sample was hereafter exposed to light (650W) for 3 min at a distance to the light source of approximately 20 cm. The RT-PCR data obtained were used to evaluate the validity of the results obtained by CFU determinations. This is the first time PMA RT-PCR is used distinction of dead and viable cells of *C. sakazakii*.

PED2.51 *L. monocytogenes* is gradually inactivated in three processed cheese analogs stored under proper refrigeration but grows under severe temperature abuse.

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The behaviour of *Listeria monocytogenes* was studied in three processed cheese analogs (PCAs) that were inoculated to contain less than 100, ca. $5x10^3$ and ca. $5x10^5$ CFU of a cocktail of three *L. monocytogenes* strains per g. The inoculated products were aseptically packaged under vacuum to mimic their respective market package conditions and stored at 4, 12 and 22°C. Based on their physicochemical characteristics (a_w and pH) and their shelf life neither of the PCAs can be automatically classified as products unable to support the growth of *L. monocytogenes* according to the definition given in Annex I of the Commission Regulation (EC) 2073/2005 and its amendment by Commission Regulation (EC) 1441/2007. The temperature range at which these products are normally transported and stored in the market is 4-8°C. The purpose of the current work was to establish whether these products can be classified as RTE foods unable to support the growth of *L. monocytogenes* at 4°C and to determine whether the pathogen has the ability to proliferate in the products under conditions of temperature abuse (12 and 22°C). For each of the 27 different experimental conditions (product, inoculum level and storage temperature) the fate of the pathogen was monitored in duplicate for a period up to 12 months according to the ISO 11290-1 and -2 protocols, resulting in a total of 54 growth/survival curves. The behaviour of the pathogen in the PCAs was product- and storage temperature-depended. Hence, whereas none of the three products supported growth of *L. monocytogenes* at 4°C, one product supported growth at 12°C and all products supported *L. monocytogenes* growth at 22°C. In the 4°C trials as well as in the 12°C-trials of two out of the three PCAs, the *L. monocytogenes* populations declined continuously, but slowly over time. As a result, in the high and medium inoculum trials, viable *L. monocytogenes* could be detected even after 12 months of storage. The results showed that the products tested do not support the growth of *L. monocytogenes* under proper refrigeration (4°C). However, every effort should be exercised to prevent post-pasteurization product contamination and product temperature abuse, because a hypothetical contamination with *L. monocytogenes* can persist in the products for a considerable time under refrigeration or can lead to pathogen outgrowth under conditions of severe temperature abuse.