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Inactivation of *Enterobacter sakazakii* by pulsed electric field in buffered peptone water and infant formula milk

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Abstract

The effect of high-intensity pulsed electric field (PEF) treatment on the survival of *Enterobacter sakazakii* suspended in buffered peptone water (BPW) and powdered infant formula milk (IFM) was evaluated. Reference medium and IFM samples were treated with PEF. Electric field intensity and treatment time were varied from 10 to 40 kV cm^{-1} and from 60 to 3895 μs, respectively. Samples of buffered peptone water (3 g L^{-1}) and IFM were inoculated with *E. sakazakii* (CECT 858) (10^9 cfu mL^{-1}) and then treated with PEF. The inactivation data were adjusted to the Weibull frequency distribution function and Bigelow model, and constants were calculated for both substrates. A maximum 2.7 log (cfu mL^{-1}) reduction was achieved in BPW after exposure of *E. sakazakii* to PEF for 360 μs (2.5 μs pulse width) at 40 kV cm^{-1}. In IFM, exposure of *E. sakazakii* to PEF, with the same conditions, led to a 1.2 log (cfu mL^{-1}) reduction. The greater the field strength and treatment time, the greater the inactivation achieved in both substrates. Even though further research will be necessary, according to the results, there are good prospects for the use of PEF in hospitals to achieve safe reconstituted infant formula before storage at refrigerated temperatures.

Keywords: Infant formula milk; Pulsed electric fields; *Enterobacter sakazakii*; Inactivation kinetics

1. Introduction

There are now many foods specifically manufactured for infants less than 1 year old and young children aged between 1 and 3. These foods which differ in compositions and manufacturing processes are intended to satisfy the specific nutritional requirements of these subgroups of the population.

Since infants and young children are known to be particularly vulnerable to food-borne infections, the microbiological safety of these products is of utmost importance.

Epidemiological studies have implicated *Enterobacter sakazakii* as an occasional contaminant of powdered infant formula milk (IFM) and this causes, on occasion, life-threatening sepsis, neonatal meningitis, bacteraemia, necrotizing enterocolitis (NEC) and necrotizing meningoencephalitis after ingestion (Muytjens & Kollee, 1990).

Urmenyi and Franklin (1961) reported the first two known cases of neonatal meningitis and septicaemia caused by *E. sakazakii*, described at that time as a yellow-pigmented *E. cloacae* strain. Jöker, Northolm, and Siboni (1965) reported an additional case of meningitis caused by a yellow-pigmented *E. cloacae* strain. In 1980, the name *E. sakazakii* was proposed for this new species by Farmer, Asbury, Hickman, and Brenner (1980).

While *E. sakazakii* has caused disease in all age groups, the great majority of cases are seen in infants less than 2 months old. Approximately, 50 cases have been reported world wide in infants less than 60 days old (Iversen & Forsythe, 2003), although under reporting is suspected. The data for these infants are incomplete, but most of them were premature (gestation <37 weeks) and had a low birth weight, below 2500 g (Codex, 2003; Lai, 2001). Although premature infants and those with underlying medical

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conditions have the highest risk of developing an *E. sakazakii* infection, a healthy, full-term, newborn infant in Iceland became ill prior to hospital discharge and suffered permanent neurological sequelae as a result of an *E. sakazakii* infection. This condition has an incidence of 2–5% in premature infants and 13% in those weighing less than 1.5 kg at birth. A mortality rate of 40–80% has been reported in the literature in neonates within days of birth (Iversen & Forsythe, 2003).

Public health authorities and researchers are exploring ways of eliminating the bacterium or controlling its growth in dry infant formula processing environments and formula preparation areas in hospitals. Skaladal et al. (1993) found *E. sakazakii* to be one of the major contaminating bacteria in ultra-high-temperature (UHT) milk cartons, implying that the organism may survive UHT temperatures or post-processing contamination may take place. Nazarowec-White and Farber (1997) reported that 0–12% of infant formula samples found in the Canadian retail market (from five different companies) contained *E. sakazakii*.

Recently, the International Commission for Microbiological Specifications for Foods (2002) ranked the organism as a "severe hazard for restricted populations, life threatening or substantial chronic sequelae or long duration". Since then it has had the same ranking as more familiar food- and water-borne pathogens such as *Listeria monocytogenes*, *Clostridium botulinum* types A and BV, and *Cryptosporidium parvum*.

Airborne *E. sakazakii* can resist the stressing environment in the processing plant, survive adhered to equipment and recontaminate powder during the handling and filling processes. Recontamination can also take place during preparation or reconstitution of the infant formula due to poorly sterilized baby bottles or poorly maintained equipment at home and in hospitals. It is probable that outbreaks are due to gross temperature abuse and/or poor hygienic practice.

In both the home and the hospital, it is of the greatest concern that recontamination and the possible growth of *E. sakazakii* should be avoided in the preparation and storage of reconstituted infant formula. In both situations, sterile water and aseptic conditions for reconstitution are essential.

Although there is no epidemiological evidence for the amount of an infectious dose, it would seem reasonable to use a detection limit <1 cell in 25 g of infant food formula, equivalent to that of detection of *Salmonella* in milk powder (Mansfield & Forsythe, 2000). Infant formula producers consider that a detection limit of 1 cfu 100 g^{-1} should be the target even for sublethally injured cells and also in the presence of large number of competitors (EFSA, 2004).

There is no evidence, however, for claiming *E. sakazakii* to be posing any significant risk to general populations consuming food products that comply with recognized international food processing or public health standards. There are only a few reports of infections among adults, and most adult patients with *E. sakazakii* infection had serious underlying diseases such as malignancies (Lai, 2001).

In view of the potential of pulsed electric field (PEF) as a friendly technology in relation to the more sensitive components of foods, research was carried out to determine the effectiveness of PEF treatments on the inactivation of *E. sakazakii* as an additional control measure to accompany good manufacturing practices (GMP) and good hygienic practices (GHP) in the production of infant formulas.

2. Material and methods

2.1. Microbiological

A pure culture of *E. sakazakii* (CECT 858 equivalent to 29522 ATCC) bacterium was provided freeze dried by the Spanish Type Culture Collection.

The culture was rehydrated in 10 mL of Tryptic Soy Broth (TSB) (Scharlab Chemie, Barcelona, Spain). After 20 min, the 10 mL was inoculated into 500 mL of TSB and incubated at 36 °C with continuous agitation at 200 rpm for 24 h to obtain cells in a stationary growth stage.

The cells were removed by centrifugation twice at 7000 rpm, 4 °C, for 15 min and then resuspended into 100 mL of TSB. After the second centrifugation, the cells were resuspended in 15 mL of TSB, and dispensed into 2-mL vials, with 1 mL of cell suspension and 1 mL of TSB solution with glycerol (20%). The 2-mL samples were immediately frozen and stored at −80 °C. These are the 15 vials of mother culture with a final concentration of 10^9 cfu mL^{-1}.

The next step consisted of a repeat of the above process taking one "mother culture vial" and diluting with TSB to obtain 10^6 cfu mL^{-1}. One milliliter of this solution was transferred into a flask containing 500 mL of TSB and incubated at 36 °C with continuous agitation at 200 rpm for 14 h to obtain cells in a stationary growth stage. Curves of cell growth versus time were obtained. Cell suspensions were centrifuged twice at 7000 rpm, 4 °C, for 15 min and the first cell pellet was resuspended into 100 mL of TSB. The second pellet was added to 50mL of TSB and 50 vials of 2 mL were prepared, each one with 1 mL of cell suspension and 1 mL of TSB solution with glycerol (20%) and a final concentration of $1–3 \times 10^9$ cfu mL^{-1}. Then the cell vials were frozen at −80 °C for use in PEF experiments (Ananta, Heinz, Schlüter, & Knorr, 2001; De Paoli, 2005; Pothakamury, Monsalve-González, Barbosa-Cánovas, & Swanson, 1995; Rodrigo et al., 2003). All experiments were completed within 2 months.

2.2. Treatment medium and inoculation

2.2.1. Buffered peptone water

Buffered peptone water at a concentration of 0.3% (w/v), with a conductivity of 2.5 mS cm^{-1} and a pH value