Food Microbiology 62 (2017) 82–91

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Drying parameters greatly affect the destruction of *Cronobacter sakazakii* and *Salmonella* Typhimurium in standard buffer and milk

Emilie Lang [a, b], Cyril Iaconelli [a], Fiona Zoz [a], Stéphane Guyot [a], Pablo Alvarez-Martin [b], Laurent Beney [a], Jean-Marie Perrier-Cornet [a], Patrick Gervais [a, *]

[a] UMR PAM A 02.102 Procédés Alimentaires et Microbiologiques, Université de Bourgogne Franche-Comté/AgroSup Dijon, 1, Esplanade Erasme, 21000 Dijon, France

[b] Novolyze, 50 Rue de Dijon, 21121 Daix, France

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form
8 February 2016
Accepted 2 October 2016
Available online 5 October 2016

Keywords:
Salmonella Typhimurium
Cronobacter sakazakii
Cultivability
Milk
Drying conditions

ABSTRACT

Salmonella Typhimurium and *Cronobacter sakazakii* are two foodborne pathogens involved in neonatal infections from milk powder and infant formula. Their ability to survive in low-moisture food and during processing from the decontamination to the dried state is a major issue in food protection. In this work, we studied the effects of the drying process on *Salmonella* Typhimurium and *Cronobacter sakazakii*, with the aim of identifying the drying parameters that could promote greater inactivation of these two foodborne pathogens. These two bacteria were dried under different atmospheric relative humidities in milk and phosphate-buffered saline, and the delays in growth recovery and cultivability were followed. We found that water activity was related to microorganism resistance. *C. sakazakii* was more resistant to drying than was *S.* Typhimurium, and milk increased the cultivability and recovery of these two species. High drying rates and low final water activity levels (0.11–0.58) had a strong negative effect on the growth recovery and cultivability of these species. In conclusion, we suggest that effective use of drying processes may provide a complementary tool for food decontamination and food safety during the production of low-moisture foods.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Salmonella enterica is a Gram-negative, facultative anaerobic, motile, nonspore-forming bacterium that causes human salmonellosis. It is a major pathogen in the food industry and is highly represented in outbreaks across the world, with nearly 100,000 cases reported every year in the European Union. Because of 10^5-10^{10} cells in adults and few microorganisms in young children and elderly people (10–100 cells) cause illness, the bacterium is important in food safety and the food industry, and must be eliminated from food products (Bhunia, 2008; Gomez et al., 1997). *Cronobacter sakazakii*, another Gram-negative, facultative anaerobic, motile, and nonspore-forming bacterium, is considered an opportunistic pathogen that can cause severe infections such as meningitis, bacteremia, and necrotizing enterocolitis in infants. However, the infective dose is not well defined and the incidence of this bacterium is largely underestimated. Death rates in *Cronobacter* infections are up to 80% and *Cronobacter sakazakii* contamination is another important issue for the infant formula industry (Yan et al., 2012).

In recent years, some reported cases have involved the presence of these two pathogens in infant formula or milk powder (Beuchat et al., 2013). Milk is pasteurized and then spray-dried to provide the low water activity (a_W) of 0.35–0.25 needed to preserve the nutritional, organoleptic, and microbial qualities over time (Beuchat et al., 2013). However, milk contamination can occur during the transfer to spray-drying and during handling of dried milk (Podolak et al., 2010). This is reflected by outbreaks involving *Cronobacter* spp., such as 3 cases in 1986 in Iceland, 4 cases in 1988 in the USA, 12 cases in 1998 in Belgium, 11 cases in 2001 in the USA, 3 cases in 2004 in France, 2 cases in 2008 in USA, and 4 cases in 2011 in USA. Examples of outbreaks involving *Salmonella enterica* include 3000 cases in 1976 in Trinidad, 76 cases in 1986 in the UK, 141 cases in 2005 in France, 42 cases in 2008 in Spain, and 16 cases in 2012 in Russia. These outbreaks involved contamination of powdered infant formula or milk powder, which are low-moisture foods (Beuchat et al., 2013; CDC, 2012, 2009; Finn et al., 2013;

* Corresponding author.
E-mail address: patrick.gervais@u-bourgogne.fr (P. Gervais).

http://dx.doi.org/10.1016/j.fm.2016.10.005
0740-0020/© 2016 Elsevier Ltd. All rights reserved.

Forsythe, 2014; Institut de veille sanitaire, 2006).

These two bacteria are found in a large range of food products and in the food industry environment (Beuchat et al., 2013; Friedemann, 2007; Iversen and Forsythe, 2004; Jaradat et al., 2009; Van Doren et al., 2013; Yan et al., 2012). a_W is a thermodynamic parameter that reflects the water availability in the environment and can affect every system involving water such as the biochemical, biological, and physiological reactions. . Bacteria have many active mechanisms, involving the activation of several resistance pathways, including sugar and amino acid accumulation, scavenger enzyme production, DNA repair, and other protein production processes, to resist dehydration caused by a change in environmental conditions, including preventing or repairing damage (Alvarez-Ordóñez et al., 2015; Billi and Potts, 2002; Deng et al., 2012; Feeney and Sleator, 2011; Gruzdev et al., 2011; Howells et al., 2002; Potts, 2001, 1994; Spector and Kenyon, 2012).

Passive physical reactions take place as water exits cells during drying. The osmotic pressure increases during the first decrease in a_W through to the end of the liquid state, and oxidative stress may occur in response to the cell's exposure to an oxygen-containing atmosphere (Dupont et al., 2014). Below an a_W threshold of ~0.90, active bacterial responses no longer occur because of the low molecular mobility and bacterial metabolism (Billi and Potts, 2002; Potts, 2001, 1994). The preserved molecules needed for bacterial metabolism and cell viability may differ according to the damage caused. Thus, determining the viability by measuring cultivability may be a restrictive way to evaluate bacterial functionality after drying (Lesn et al., 2000; Spector and Kenyon, 2012).

Microorganisms are more resistant to the thermal decontamination process when in the dried state compared with the liquid state (Arroyo et al., 2012; Fine and Gervais, 2005; Laroche and Gervais, 2003; Laroche et al., 2005; Rychlik and Barrow, 2005; Skandamis et al., 2008). This particular resistance is largely attributed to the low molecular mobility (Farakos et al., 2013; Lian et al., 2015), and also to the cross-protection occurring during the transition to the dried state (Finn et al., 2013; Gruzdev et al., 2011; Shen and Fang, 2012). Inactivation of pathogens in low-a_W food is too complicated for accurate prediction because of the complex effects on cultivability (Fine et al., 2005; Finn et al., 2013). Because the drying process is involved in causing bacterial death, drying may be a potential kill step during the handling of dried products.

Because of the danger for infants and the difficulty in eliminating, in dried state, bacterial pathogens such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Cronobacter sakazakii*, the drying process is an important step in the control of these pathogens. From this perspective, this study aimed to understand the effects of drying parameters on these two pathogens. Three parameters were controlled: drying substrate, atmospheric relative humidity (RH), and final a_W. To observe the effects of drying on bacterial cells, cultivability after treatment was measured and the growth delay and cell recovery capacity were estimated. This provided an indirect measure of the deleterious effects on and repairable damage to the bacteria.

2. Materials and methods

2.1. Bacteria species

Salmonella enterica subspecies *enteric*a serovar Typhimurium DT104 DSM 10506 and *Cronobacter sakazakii* CIP 103183T species were used. All cultures were stored in Tryptic Soy Broth (TSB, Sigma-Aldrich) with 20% glycerol (Sigma-Aldrich, Saint-Quentin-Fallavier, France) at −80 °C. For resuscitation, bacteria were inoculated on Tryptic Soya Agar (TSA, Sigma-Aldrich) at 37 °C for 24 h and were placed for a maximum of one month at 4 °C. Five colonies of each bacterium were collected in 50 mL of TSB and incubated for 8 h at 37 °C. Suspensions were then diluted in 50 mL of new TSB to reach an optical density (OD) of 0.01 at 600 nm. Cultures in stationary growth phase were obtained after 14 h at 37 °C.

2.2. Drying conditions

2.2.1. Drying atmosphere

Hermetic boxes were used to dry the cellular suspensions. Saturated salt solutions were placed at the bottom to regulate the a_W and the atmospheric RH. Lithium chloride, potassium acetate, potassium carbonate, and sodium bromide (all from Sigma-Aldrich) were used in sufficient quantity to observed salt crystals in 100 mL of distilled water, corresponding to a height of 2 cm in the bottom of the hermetic boxes. Their respective a_W values were 0.11, 0.25, 0.44, and 0.58, which corresponded to RH values of 11%, 25%, 44%, and 58%, respectively (Greenspan, 1977). The atmosphere was maintained under convection using a ventilator (Sunon, Radiospare, France) as described in previous work (Lemetais et al., 2012). All experiments were performed at room temperature (near 25 °C).

2.2.2. Drying kinetics of PBS and milk by following the weight loss

The drying of phosphate-buffered saline (PBS, Sigma-Aldrich) and whole milk from milk powder that was rehydrated by following the manufacturer instructions (10% w/v, 26% fat, Regilait, Saint-Martin-Belle-Roche, France) was monitored by measuring the weight loss over time. The weight was measured with a precision balance (±0.0003 g) (Sartorius, Aubagne, France). The measured weight loss followed the first-order law (Eq. (1)). Using the reduction in the sum of squares scores between the experimental curve and the model (*fminsearch* function in Matlab R2012a), we estimated three variables: *a*, *K*, and *τ*. These provided a characterization of the drying rate.

$$w_t = a + K\exp\left(\frac{-t}{\tau}\right) \quad (1)$$

where w_t (g) represents the weight at time t (s), a represents the dried weight (g), K represents the initial water weight (g), and τ is the time constant (s). The initial drying rates were calculated as a derivation of w_t at $t = 0$ s.

Because only water evaporated, this loss of weight was translated into water content (grams of water per gram of dried material), and its relationship with the sorption curve of PBS or milk was used to evaluate the a_W evolution of the sample during drying. The PBS sorption curve was obtained by measuring the water content corresponding to several a_W values. The milk desorption curve was based on an equation given in a previous work (Langová et al., 2012), which allowed us to calculate the water content corresponding to each a_W value.

2.2.3. Preparation of the initial cellular suspension

Cell concentration was adjusted to 10^8 colony-forming units (CFU)/mL by measuring OD_{600} and according to calibration curves showing the link between OD_{600} and cell concentration in CFU/mL. Twenty-five-milliliter cultures were centrifuged (3400 × *g*, 10 min at 25 °C) and washed twice with an equal volume of PBS. The supernatant was removed, and the cell pellet was resuspended in 25 mL of PBS. The final bacterial number was recorded as the number of CFU by plate counting on TSA (24 h, 37 °C).

2.2.4. Drying in PBS

Droplets (10 μL containing around 10^6 bacterial cells) of bacterial suspension were spread in a small glass dish to obtain an estimated 14 μm height layer, and the dish was placed inside a box

Download English Version:

https://daneshyari.com/en/article/4362537

Download Persian Version:

https://daneshyari.com/article/4362537

Daneshyari.com