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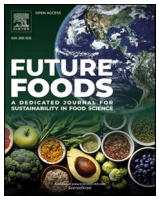

Showcasing the potential of Pulse Spray Drying for infant foods from the food safety perspective

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ABSTRACT

This study evaluated *Pediococcus acidilactici* ATCC 8042 as a non-pathogenic surrogate for *Salmonella* to assess the effect of a new Pulse Spray Drying (PSD) technology in infant cereal-based food (CBF) and powdered infant formula (PIF). The pathogen *Salmonella enterica* serovar Senftenberg ATCC 43845 was used for comparison. Thermal inactivation kinetics were determined for both organisms in CBF and PIF at three a_w levels. Subsequently, *P. acidilactici* was inoculated into both matrices and subjected to PSD at varying outlet temperatures. Microbial inactivation and hydroxymethylfurfural (HMF) and acrylamide formation were measured. A statistically significant linear correlation was found between the $LogD_T$ values of *P. acidilactici* and *Salmonella* across all conditions. The surrogate consistently showed higher thermal resistance, with D_T values averaging 4.42 times greater than those of the pathogen. These findings support the use of *P. acidilactici* ATCC 8042 as a conservative surrogate for *Salmonella*. PSD achieved microbial reductions of approximately 2–4 log, depending on outlet temperature. Levels of HMF and acrylamide were comparable to those reported in commercial products. These findings underscore PSD's potential as a safe and effective drying technology for infant foods, offering a valuable tool for food industry aiming to improve microbial safety without compromising product quality.

1. Introduction

Spray drying is the most used technology to dry liquid foods (Rodríguez Arzuaga et al., 2022). However, conventional spray drying face several challenges, including low energy efficiency, high energy demand, low powder recovery rates and low viscosity range (up to 500 mPa·s) (Baker and McKenzie, 2005; Cheng et al., 2018; Samborska et al., 2022).

As a potential alternative, Pulse Spray Drying (PSD) is an innovative drying technology that uses an engine to generate hot air waves (3000 waves per minute, at 350–400 °C). This process enables rapid drying of liquid droplets, producing high-quality dried products without the gas issues associated with traditional direct heating systems (Dantas et al., 2024; Meng et al., 2016; Wu et al., 2015). Another advantage of PSD is the possibility of working with relatively high viscosity matrixes (up to 16,000 mPa·s) making it feasible to handle semiliquid mixtures (Kudra, 2008). Moreover, the energy efficiency of PSD (2604 kJ/kg of water evaporated) was considered better than in traditional spray dryers (4500–11,500 kJ/kg of water evaporated) (Wu et al., 2015). Previous studies have explored the effect of PSD on the technological and sensory properties in various food matrixes and ingredients (e.g. egg white, skimmed milk, whey protein isolate, vegetable beverages) focussing on parameters such as moisture, solubility indexes, bulk and tapped density, particle size and/or energy efficiency (Dantas et al., 2024, 2025; Pramudita et al., 2021, 2022; Romo et al., 2024; Wu et al., 2015). Dantas et al. (2024) also employed CALUX® bioassays, which are sensitive cell-based screening tools used to detect the presence of cytotoxic, xenobiotic or genotoxic activity, thereby providing insights into the chemical safety profile of the PSD milk. These works have demonstrated that PSD could be a promising new drying technology without compromising the technological quality and toxicological safety of the product. However, to our knowledge, no published studies have evaluated the impact of PSD on relevant bacterial pathogens and process induced chemical hazards. This gap is particularly relevant given the stringent safety requirements for food intended for infants, who represent a highly vulnerable population. Investigating how PSD influences

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both microbiological safety and the formation of process induced contaminants would provide valuable insights in the food safety potential of this emerging technology to support its future application in the infant food sector.

Cereal-based food (CBF) and powdered infant formula (PIF) are important food products to sustain infant growth within the first year of life (Arku et al., 2008). Despite their shelf-stability, CBF and PIF are low-moisture foods frequently involved in recalls and outbreaks due to contamination with *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) and *Salmonella* (Donaghy et al., 2025; WHO, 2006; Yeak et al., 2024), which should not be detected in 10 and 25 g, respectively, of products placed on the market during their shelf-life (European Commission, 2005). The food safety of these products must be guaranteed through raw materials quality, good manufacturing practices and the production process (i.e. the thermal treatments associated with pasteurization of the feed solution and subsequent drying).

The impact of processing technologies on the fate of relevant microbial hazards can be assessed by challenge tests with relevant pathogens when biosafety risk measures can be applied (NACMCF, 2010). However, the use of pathogens at pilot or industrial plant scales has strict safety limitations (Hu and Gurtler, 2017; Niebuhr et al., 2008; Ozturk et al., 2020). As a feasible alternative, non-pathogenic surrogates can be used. Before using a surrogate, it is important to characterize the behavior of both surrogate and pathogenic microorganisms in each matrix and relevant processing conditions. Several studies have reported that *Enterococcus faecium* is a suitable surrogate for *Salmonella* to assess the effect of thermal processes in many low-moisture foods such as almond (Stevenson et al., 2014), fine ground black pepper (Wason et al., 2022), dried basil leaves (Verma et al., 2021) or wheat flour (Liu et al., 2018). Despite *E. faecium* is non-pathogenic, it is not a GRAS (Generally Regarded as Safe) microorganism and it does not have the QPS (Quality Presumption of Safety) status (EFSA, 2007; Hanchi et al., 2018). Using a GRAS microorganism as a surrogate such as *Pediococcus acidilactici* may have the potential to be safely used in a food facility for in-plant assessments. *P. acidilactici* has been used as surrogate for pathogenic microorganisms such as *Salmonella* spp. and *Cronobacter* spp. in food-grade galactooligosaccharides with low water activity (a_w of ca. 0.7) heated between 70 and 85 °C (Bang et al., 2017), and in dry pet food heated between 76.7 and 87.8 °C (Ceylan and Bautista, 2015). To our knowledge, *P. acidilactici* has not previously been qualified as a surrogate for *Salmonella* to assess the impact of drying on CBF or PIF.

Concerning chemical hazards, process-induced contaminants have been described to be present in commercial infant foods, such as 5-hydroxymethylfurfural (HMF) in PIF, and acrylamide in CBF (Boyaci-Gunduz, 2022; Chávez-Servín et al., 2015; Cui et al., 2020; Mojska et al., 2012; Vella and Attard, 2019). HMF forms when sugars are heated, particularly through the Maillard reaction, and is commonly found in processed foods like honey, fruit juices, and baked goods. In commercial PIF, HMF content varies from 0.06 to 7.87 mg/kg (Cui et al., 2020; Vella and Attard, 2019). Its potential mutagenic and genotoxic effects raise concerns about its presence in infant foods. Spray drying technology is known to affect the amount of HMF formed, linked to the outlet temperature. For instance, powdered milk obtained by conventional spray drying showed about 14 % higher HMF contents than powders from electrostatic spray drying and freeze-drying (Chutani et al., 2024). Additionally, HMF serves as an indicator of quality, often resulting from overheating or prolonged storage in carbohydrate-containing foods (Gökmen and Morales, 2014; Rada-Mendoza et al., 2002). Acrylamide primarily forms in carbohydrate-rich foods during high-temperature cooking processes, such as frying, baking, and roasting, through Maillard reaction (Halford and Curtis, 2019). These chemical hazards, generated during thermal processing like drying, pose significant risks, especially for vulnerable populations such as infants (Boyaci-Gunduz, 2022). Data collected from European member states showed acrylamide contents in processed cereal-based infant foods averaging 74 μg/kg with the percentile 95th at 175 μg/kg and maximum levels at 353 μg/kg, representing 30 % of the lower bound average chronic dietary exposure in some infant groups (EFSA, 2009; EFSA CONTAM Panel, 2015). As PSD technology enables a more rapid drying process compared with conventional spry drying, the formation of HMF and acrylamide could potentially be reduced. However, to our knowledge, no study has addressed the impact of PSD on the formation of these process-induced contaminants in PIF and CBF.

In this framework, this study aimed to evaluate the impact of PSD on key microbiological and chemical hazards in two types of infant foods: PIF and CBF. Due to the biosafety constraints preventing the use of pathogenic bacteria in the pilot plant PSD equipment, a laboratory scale study was first conducted to qualify *P. acidilactici* ATCC 8042 as a non-pathogenic surrogate for *Salmonella* spp. across different water activities (a_w) in PIF and CBF. Subsequently, the impact of PSD conditions on microbial inactivation was evaluated in both matrices inoculated with the qualified surrogate. Additionally, the formation of process-induced contaminants, such as HMF in CBF and PIF and acrylamide in CBF, was quantified under different PSD conditions.

2. Material and methods

2.1. Qualification of *P. acidilactici* as non-pathogenic surrogate of *Salmonella spp.*

2.1.1. Bacterial culture preparation

Salmonella enterica serovar Senftenberg ATCC 43845 was used as pathogen. This strain exhibits increased heat tolerance and it is commonly used as worst-case scenario in heat resistance studies (Nguyen et al., 2017). The strain *P. acidilactici* ATCC 8042 was selected as potential surrogate with GRAS and QPS status. Each bacterium was independently cultured in Brain Heart Infusion broth (BHI) (Beckton Dickinson, Sparks, MD, USA) for 8 h at 37 °C and subsequently sub-cultured in BHI for 18 h at 37 °C. These cultures were frozen at −80 °C in the growth medium supplemented with 20 % glycerol as cryoprotectant until use. Frozen cultures were used because freezing conditions expose microbial cells to concentrated solutes, leading to osmotic stress conditions similar to those caused by dry environments occurring in the food industry (e.g. food contact surfaces, dry ingredients). This type of stress is known to trigger protection against physical treatments such as temperature (including drying) and high pressure processing (Hereu et al., 2014; Syamaladevi et al., 2016; Wesche et al., 2009; Wilson et al., 2012; Xu et al., 2019). Moreover, working with stock cultures kept at −80 °C enables to keep harmonised conditions across the inoculation experiments and control the variability due to differences on the physiological state of the bacterial culture.

2.1.2. Infant food matrix preparation

CBF and PIF were prepared from the ingredients provided by Yiotis S. A. (Athens, Greece), following recipe indicated by this industrial infant food manufacturer. Briefly, CBF consisted of wheat flour (9 %), starch (6 %), sugar (6 %), whole milk powder (6 %), skimmed milk powder (4 %), and whey protein (4 %). The ingredients for PIF included lactose powder (20.5 %), whole milk powder (13.7 %), vegetable fat (11.7 %), and whey protein powder (8.5 %), targeting a whey:casein ratio to mimic human milk (commonly 60:40).

The thermal resistance of the pathogen and surrogate in PIF and CBF was evaluated for 3 different a_w levels: low-a_w (dry powdered CBF and PIF showing a a_w of 0.45 ± 0.02); mid-a_w (powder equilibrated to a_w of 0.75 ± 0.01 in sealed containers containing 65 % sodium chloride and 30 % potassium chloride saturated solutions for 24 h) and high-a_w (rehydrated powder with an a_w of 0.98 ± 0.002). Values of a_w were verified at 25 °C using an Aqualab® equipment (Decagon Devices, Pullman, WA, USA).

2.1.3. Inoculation and treatment

The cultures (1 mL) were thawed and centrifuged (D3024, DLAB

Scientific Co., Ltd., Beijing, China) at 13,000 rpm for 15 min at room temperature. The supernatant was discarded, and the pellet was mixed with 1 g of sterile sand (PanReac AppliChem, Castellar del Vallès, Spain) as dry inoculum carrier. The inoculated sand was mixed at 1:99 ratio in CBF or PIF matrix to achieve a target inoculation level of 7 log CFU/g. For PIF, the vegetable fat was added after the inoculated sand and mixed by hand. The inoculated powders were split (2 g aliquots) into autoclave plastic-pouches (Sarstedt AG&Co.KG, Nümbrecht, Germany) and heat sealed removing the air.

The sample pouches were submerged in a preheated water bath (Memmert GmbH + Co. KG, Schwabach, Germany) at different temperature and time combinations ranging from 60 to 90 °C up to 30 min. The sample pouches were pulled at predetermined time intervals once the product had reached target temperatures and immediately placed in an ice-water bath for at least 30 s to stop the thermal treatment. Unheated inoculated samples were used to establish the initial microbial count in the samples. Three independent replicates for each temperature and time combination were carried out.

2.1.4. Bacterial enumeration

Samples were transferred from pouches into 50-ml sterile tubes and diluted 1:10 with saline solution (8.5 g/L sodium chloride, 1 g/L bacterial peptone). Appropriate serial dilutions of *Salmonella* spp. and *P. acidilactici* inoculated samples were spread-plated in Chromagar Salmonella and Man-Rogosa-Sharpe (MRS) agar (Merck, Darmstadt, Germany), respectively. The plates were incubated for 24 h at 37 °C and 2-3 days at 30 °C, respectively, for colony enumeration. Log reductions were calculated by subtracting log survivor counts of either microorganism (Log N) from the initial log population (Log N_0).

2.1.5. Thermal resistance of bacteria

The thermal resistance parameters of the pathogen (*Salmonella*) and the potential non-pathogenic surrogate (*P. acidilactici*) were determined in terms of decimal reduction time (i.e. D_T value as the time to achieve 1 log reduction) and thermal resistant constant (i.e., z value as the temperature increase required to reduce the D_T value by a factor of 10).

The D_T value associated with each temperature was estimated by fitting the log-linear inactivation model (Eq. (1)) to each kinetic dataset (log survivors along the treatment time) for *P. acidilactici* and *Salmonella* in CBF and PIF with different a_w.

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D_T} \tag{1}$$

Where N_0 and N (CFU/g) are the initial counts before treatment and the counts at a given treatment time (t, min), and D_T is the D_T value (min) at a given temperature (T). Model fitting was performed using the Add-in GInaFit freeware tool for Microsoft Excel (Geeraerd et al., 2005), which provides parameter estimates and the goodness-of-fit indexes, i.e., the residual sum of squares (RSS), the root mean squared error (RMSE), and the coefficient of determination (R^2).

Secondary modelling using Bigelow model (Eq. (2)) was performed to estimate the z value, as the parameter quantifying the impact of temperature on the D_T value. The model fitting was performed for each matrix and a_w value.

$$LogD_T = LogD_{Tref} + \frac{T_{ref} - T}{z} \tag{2}$$

Where D_{Tref} is the decimal reduction time (min) at T_{ref}, T_{ref} is the reference temperature (70 °C), T is the treatment temperature (°C), z is the temperature increment needed to reduce the D_T value by a factor of 10. Secondary model fitting was carried out with the MS Excel Solver Add-in.

The relationship between thermal resistance of *Salmonella* and *P. acidilactici* was assessed through linear regression of the respective LogD_T values with JMP version 16 (SAS institute, Cary, NC, USA). Moreover, the bias factor (B_f) was calculated according to Eq. (3), allowing to quantify the average systematic bias between the thermal resistance behaviour of the compared bacteria.

$$B_f = 10^{\left(\frac{\sum_{i=1}^{n} LogD_{surrogate} - LogD_{pathogen}}{n}\right)} \tag{3}$$

Where $D_{surrogate}$ and $D_{pathogen}$ are the averaged decimal reduction time (D_T, min) observed at each treatment temperature and a_w combination for *P. acidilactici* and *Salmonella*, respectively. A B_f close to 1 indicates that the surrogate behaviour closely mimics that of the pathogen, making it a reliable stand-in for validation studies. Significant deviations from 1 suggest discrepancies that need to be addressed. When B_f is higher than 1, the surrogate tends to show higher thermal resistance (longer D_T values) compared to the pathogen; while B_f lower than 1 indicates that the surrogate is more thermo-sensitive compared to the pathogen.

2.2. Pilot plant experiments with PSD processing

2.2.1. Bacterial culture preparation

P. acidilactici ATCC 8042 was cultured (1 %) in Brain Heart Infusion (BHI) (Beckton Dickinson, Sparks, Md., USA) at 30 °C overnight and, subsequently, sub-cultured (1 %) at 30 °C overnight with agitation. The culture was centrifuged (Beckman Coulter, Brea, CA, USA) for 6 min at 6,000 rpm. Bacterial mass (pellet) was resuspended in cryoprotectant solution composed of 3 g/L beef extract, 5 g/L triptone and 200 g/L glycerol in distilled water, to reach a concentration of *ca.* 11 log CFU/mL, and stored at −80 °C until use.

2.2.2. PSD feed matrix preparation and experimental design

For CBF, the ingredients (9 % wheat flour, 6 % starch, 6 % sugar, 6 % whole milk powder, 4 % skimmed milk powder and 4 % whey protein, following a standard recipe indicated by Yiotis S.A., Greece) were reconstituted with distilled water at 70 °C resulting in 35 % of total solids. To hydrolyse the starch, calcium chloride (0.25 g/kg dry weight) and BIALFA CA enzyme (α-amylase, EC: 3.2.1.1, CYGYC BIOCON, S.L., Spain) was added (0.2 % dry weight). After 30 min, the CBF solution was homogenized (BERTOLI HA31002, Interpump Group S.p.A., Sant'Ilario d'Enza, Italy) to achieve particle size reduction and improve emulsion stability. The homogenization was carried out in a double-stage homogenization system at a total pressure of 240 bar (200 + 40 bar). Then, the homogenized CBF was pasteurized (90 °C, 11.45 s) in a UHT system (ISI00-328468001 model, INOXPA S.A.U., Banyoles, Spain) to inactivate the enzyme and eliminate the background microbiota. Finally, the CBF feed product was cooled to 30 °C.

PIF ingredients (15.2 % lactose powder, 10 % whole milk powder, 8.6 % vegetable fat, 6.2 % whey protein powder, all provided by Yiotis S.A., Greece) were reconstituted with distilled water at 70 °C resulting in 40 % of total solids. The reconstituted PIF was homogenized in a double-stage homogenization system at a total pressure of 180 bar (150 + 30 bar) and pasteurized (78 °C, 11.45 s) in a UHT system to eliminate the background microbiota. Finally, the PIF feed product was cooled to 30 °C.

Before PSD, *P. acidilactici* ATCC 8042 was inoculated into the CBF and PIF products at a final concentration of *ca.* 6 log CFU/g and homogenized for approximately 30 min. Drying by PSD (PCD-70, Ekonek, Errenteria, Spain; evaporation capacity of 70 kg/h) was performed under a constant propane inlet flow at 4.7 kg/h generated in the combustion motor at 148 Hz. CBF product was dried at two different outlet temperatures (95 and 110 °C), while PIF was dried at 80, 90 and 100 °C, representative of the temperature conditions applied for commercial manufacture of CBF and PIF. For PIF, the feed flow rates were set at 81 L/h, 67 L/h, and 50 L/h, corresponding to outlet temperatures of 80 °C, 90 °C, and 100 °C, respectively. For CBF, the feed flow rates were 50 L/h and 37 L/h, associated with outlet temperatures of 95 °C and 110 °C,

respectively. The feed product was dispersed using nozzle N14 (Ekonek, Errenteria, Spain) and an atomizing air pressure of 3 bar. The output dried products (powder) were collected in polypropylene boxes located under the rotary valves of the main chamber and at the end of the cyclone separator. For each treatment, the resulting powder samples were collected at three different times during the same drying batch (at 30 and 45 min) from both the main chamber (primary powder) and the cyclone (secondary powder). Two independent trials for each type of infant food were carried out.

2.2.3. Enumeration of P. acidilactici

Feed product and powder samples were 10-fold diluted in saline solution, homogenized for 30 s with a vortex agitation, 10-fold serially diluted in saline solution, plated onto MRS agar (Merck, Darmstadt, Germany) and incubated in anaerobic conditions at 30 °C for 72 h. Microbial counts of powder samples were calculated taking into account the reconstitution of CBF (1.75 g of CBF powder per 5 g of reconstituted CBF) and PIF (2 g of PIF powder /5 g of reconstituted PIF) when preparing the feed product. Microbial inactivation after PSD was expressed as bacterial log reductions, which were calculated by subtracting the concentration of survivors after each treatment (log CFU/g) from the initial concentration of (inoculated) bacteria in the feed solution (log CFU/g).

2.2.4. Determination of process-induced contaminants

HMF quantification was carried out in PIF and CBF powder samples, while acrylamide in CBF samples. HMF and acrylamide determinations were performed as described by Gökmen & Şenyuva (2006) and Ross & Williams (2019). For HMF, samples (1 g) were transferred to polypropylene tubes of 50 mL and 100 μL of Carrez I and Carrez II (Panreac Quimica S.L.U., Barcelona, Spain) and 9.8 mL of ultrapure water with 0.2 mM of acetic acid (Merck, Darmstadt, Germany) were added and vortexed for 3 min. After centrifugation for 15 min at 11,750 rpm and 0 °C (Beckman Coulter, Brea, CA, USA), the supernatant was filtered through 0.45 μm nylon filters (UniPrep, Whatman, Maidstone, UK) and cleaned by solid phase extraction (SPE) using OASIS Prime HLB cartridges (Waters, Milford, MA, USA) conditioned with 1 mL of methanol (Merck, Darmstadt, Germany) and equilibrated with 1 mL of ultrapure water. The cartridges were loaded with 1 mL of extract, washed with 2.5 mL of ultrapure water, dried under vacuum and eluted with 2.5 mL of diethyl ether. The eluate was evaporated under nitrogen at 40 °C and reconstituted in 1 mL of ultrapure water. The reconstituted extracts were centrifuged for 15 min at 13,800 rpm and 4 °C (Eppendorf, Hamburg, Germany) and the supernatant filtered using 0.2 μm nylon filters (Agilent Technologies) in chromatographic vials (Waters, Milford, MA, USA) before analysis by UPLC-MS/MS. The chromatographic system consisted of an Acquity Premier UPLC, equipped with a diode array detector, an electrospray (ESI) as ionization source and a TQ-S micro triple quadrupole mass spectrometer detector (Waters, Milford, MA, USA). The system was controlled by MassLynx 4.2 software (Waters, Milford, MA, USA). Chromatographic separation was achieved in a 100 × 2.1 mm, 1.8 μm particle size HSS T3 column (Waters, Milford, MA, USA) at 40 °C with a flow rate 0.45 mL/min and sample injection volume of 5 μL. The mobile phase followed a linear gradient between A (ultrapure water with 0.1 % of formic acid) and B (acetonitrile 100 %) as follows: 0.0 min 100 % A, 1.0 min 100 % A, 6.0 min 40 % A, 6.1 min 0 % A, 12.0 min 0 % A, 12.1 min 50 % A, 13 min 100 % A with a total run of 17 min. The source temperature was fixed at 150 °C, the capillary voltage at 0.5 kV and desolvation temperature at 500 °C. The source gas desolvation flow was 1000 L/h and 50 L/h for the cone. The cone voltage was set at 24 V. HMF was quantified with an external calibration curve employing MS/MS with multiple reaction monitoring using specific transitions (127 m/z-> 53 m/z quantification transition and 127 m/z -> 109 m/z qualification transition).

For acrylamide, 1 g sample aliquot was transferred to polypropylene tubes of 50 mL, an internal standard solution (acrylamide-d3, Merck, Darmstadt, Germany) was added and mixed. Five mL of hexane (Merck, Darmstadt, Germany), 10 mL of ultrapure water (Milli-Q system, Millipore, Bedford, MA, USA) and 10 mL of acetonitrile (Merck, Darmstadt, Germany) were added and vortexed for 3 min. Subsequently, DisQUE QUECHERS (Waters, Milford, MA, USA) were added and mixed for 1 min. Tubes were centrifuged for 15 min at 10,000 *g* and 4 °C (Beckman Coulter, Brea, CA, USA). The hexane was discarded, and 2 mL of the acetonitrile supernatant was taken for further clean-up by solid phase extraction (SPE) using OASIS Prime MCX (Waters, Milford, MA, USA) cartridges conditioned with 2 mL of methanol (Merck, Darmstadt, Germany), loaded with 2 mL of acetonitrile supernatant and eluted with 0.5 mL of ultrapure water. The eluates were evaporated under nitrogen stream at 40 °C and reconstituted in 1 mL of ultrapure water with 0.1 % of formic acid (Merck, Darmstadt, Germany) by vortexing for 1 min. The reconstituted extracts were centrifuged for 15 min at 13,800 rpm and 4 °C (Eppendorf, Hamburg, Germany) and the supernatant filtered through 0.2 μm nylon filters (Agilent Technologies, Sta. Clara, CA, USA) in chromatographic vials (Waters, Milford, MA, USA) before analysis by UPLC-MS/MS. Chromatographic separation was achieved in a 2.1 × 100 mm, 1.8 μm particle size HSS C18 SB column (Waters, Milford, MA, USA) with the proper Van guard precolumn 2.1 × 5 mm. The column temperature was 30 °C, the flow rate 0.2 mL/min and the sample injection volume 5 μL. The mobile phase followed a gradient between A (0.1 % formic acid) and B (methanol 100 %) as follows: 0.0 min 99 % A, 4.0 min 95 % A, 4.5 min 10 % A, and 8.0 min 10 % A, 8.1 min 10 % A in a 12 min total run. The TQS-micro worked with a source temperature at 150 °C, capillary voltage at 0.5 kV and desolvation temperature at 500 °C. The source gas desolvation flow was 1000 L/h and 50 L/h for the cone. The cone voltage was set at 20 V. Acrylamide was determined by MS/MS with multiple reaction monitoring using specific transitions (Acrylamide 72 m/z->55 m/z quantification transition, 72 m/z -> 27 m/z qualification transition and acrylamide-d3 75 m/z->58 m/z).

2.2.5. Statistical analysis

The results were analysed by ANOVA and Tukey's multiple comparison test with JMP version 16 (SAS institute, Cary, NC, USA). Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Thermal resistance of P. acidilactici and Salmonella as a function of temperature and a_w

The thermal resistance of the assessed bacteria is shown in Fig. 1, which depicts the log transformed D_T values (i.e., time required to reduce 1 log the bacterial load) at each assessed temperature for each infant food matrix (CBF and PIF) at different a_w.

Generally, the strain of *P. acidilactici* exhibited higher thermal resistance compared to the *Salmonella* Senftenberg strain, i.e., requiring higher temperature or longer treatment times to achieve 1 log reduction. The exception was observed in reconstituted CBF and PIF (showing high a_w) at the least intense treatments (< 70°C). In this respect, *Salmonella* Senftenberg is recognized in scientific literature as a particularly heat-resistant *Salmonella* serovar in high moisture foods (Humphrey et al., 1990; Ng et al., 1969; R. B. Read et al., 1968; Sekhon et al., 2020). However, as a_w decreases, its thermal resistance also decreases, becoming similar to that of *S.* Enteritidis or *S.* Typhimurium (Sekhon et al., 2020).

In dry CBF (with low a_w, 0.45) *P. acidilactici* showed higher ($p < 0.05$) thermoresistance than *Salmonella*. For instance, at 60 - 65 °C, the obtained D_T value exceeded 100 min for *P. acidilactici* and remained below 5 min for *Salmonella*. By increasing the temperature, the thermal resistance decreased significantly ($p < 0.05$). When the temperature was increased from 70 to 80 °C, the obtained D_T values of *P. acidilactici* and *Salmonella* dropped from 16.5 ± 6.3 and 3.7 ± 2.2 min to 4.1 ± 1.4 and 1.2 ± 0.2 min, respectively. At 90 °C, both microorganisms showed a

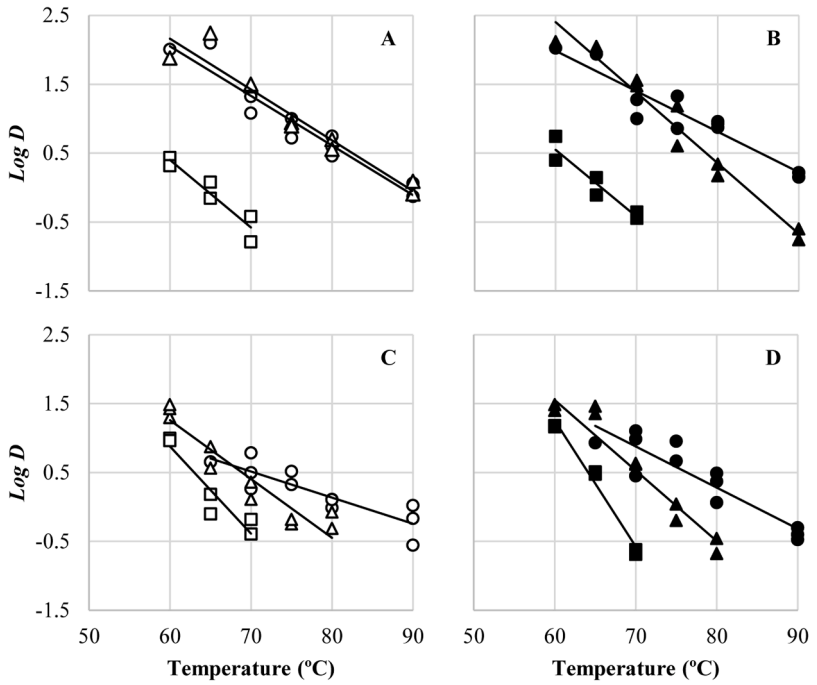

Fig. 1. Thermal resistance (log transformed D_T as a function of temperature) of *P. acidilactici* ATCC 8042 (A, B) and *Salmonella enteritidis* serovar Senftenberg ATCC 43845 (C, D) in cereal-based food (empty symbol; A, C) and powdered infant formula (full symbol; B, D) with different a_w (0.45 (●); 0.75 (▲); 0.98 (■)).

rapid inactivation kinetics with less than 1 min to achieve 1 log reduction. For PIF at a_w 0.45, similar behaviour was observed. The obtained D_T value for *P. acidilactici* and *Salmonella* (60 to 90 °C) ranged from 106.4 to 1.5 min and 8.6 to 0.4 min, respectively. These findings agreed with other studies dealing with low-moisture food. For instance, *Salmonella* (cocktail of *S. enterica* serovars Montevideo 488275, *S.* Mbandaka 698538, *S.* Agona 447967, *S.* Tennessee K4643, and *S.* Reading Moff 180418) in black pepper (a_w 0.4) significantly decreased the D_T value from 20.4 to 3.9 min when temperature increased from 70 to 80 °C (Wason et al., 2022) and from 46.0 to 7.8 min as temperature increased from 65 to 75 °C (Wei et al., 2021). Smith et al. (2016) reported decreasing D_T values for *Salmonella* Enteritidis in wheat flour (a_w 0.43) of 10.0 ± 0.5 min, 5.5 ± 0.2 and 2.1 ± 0.1 min, at 75, 80 and 85 °C, respectively.

For all matrixes and microorganisms, D_T values decreased with increasing temperature following the typical log-linear trend (Bigelow model), which allowed to estimate the *z* parameter (thermal resistance constant), for *P. acidilactici* and *Salmonella* in CBF and PIF at different a_w levels (Table 1).

The a_w of the food matrix significantly impacted the thermal resistance parameters (D_T and *z*) of both microorganisms. In general, an increase in a_w resulted in shorter D_T values. For *P. acidilactici*, this trend was statistically significant only in the rehydrated products (a_w 0.98), compared to the dry (a_w 0.45) and humidified (a_w 0.75) CBF and PIF. Specifically, for CBF (Fig. 1A), D_T values at a_w 0.45 were comparable to those at a_w 0.75 across all tested temperatures. In the case of PIF (Fig. 1B), D_T values at a_w 0.45 were similar to those at a_w 0.75 at temperature up to 75 °C; while at higher temperature, the lower a_w (0.45) resulted in higher D_T values. For *Salmonella*, the impact of a_w on the D_T values was temperature-dependent (Fig. 1C and 1D). At low temperatures (i.e., 60 °C), no significant difference in the thermoresistance of *Salmonella* was observed with respect to the a_w of the product. At higher temperatures, the effect of a_w was more evident, making the pathogen more sensitive at increasing a_w values. These observations are consistent with prior studies highlighting that the low a_w significantly protect microorganisms from thermal effects (Santillana Farakos et al., 2013;

Table 1
Thermal resistance parameter estimates of the Bigelow model for *P. acidilactici* ATCC 8042 and *Salmonella enterica* serovar Senftenberg ATCC 43845 in cereal-based food (CBF) and powdered infant formula (PIF) with different a_w, including the goodness of fit indexes[a].

Matrix	Microorganism	a_w	Log D_{Tref} (Log min)	*z* (°C)	RSS	RSME	R^2_{adj}
CBF	*P. acidilactici*	0.45	1.33	13.88	0.368	0.202	0.926
		0.75	1.42	13.56	0.366	0.214	0.928
		0.98	-0.58	10.19	0.110	0.166	0.897
	Salmonella	0.45	0.51	26.38	0.373	0.193	0.760
		0.75	0.41	11.71	0.481	0.231	0.900
		0.98	-0.39	7.90	0.189	0.217	0.895
PIF	*P. acidilactici*	0.45	1.40	17.03	0.389	0.208	0.887
		0.75	1.39	9.70	0.377	0.205	0.962
		0.98	-0.42	10.29	0.102	0.160	0.903
	Salmonella	0.45	0.88	16.64	0.580	0.254	0.808
		0.75	0.53	9.77	0.559	0.249	0.917
		0.98	-0.58	5.46	0.074	0.136	0.978

[a] RSS: Residual Sum of Squares; RMSE: Root mean sum of squared error; R^2_{adj}: adjusted coefficient of determination

Smith et al., 2016; Verma et al., 2021). In dried basil leaves processed at 75 °C, as the a_w decreased from 0.7 to 0.4, the D_T value increased from 3.3 to 9.1 min for a *Salmonella* cocktail (Agona, Tennessee, Mbandaka, Montevideo, and Reading) and from 6.5 to14.1 min for *E. faecium* (Verma et al., 2021). Similarly, in wheat flour treated at 75 °C *S.* Enteritidis increased its D_T values from 3.3 ± 0.2 at a_w 0.70 to 10.0 ± 0.5 min at a_w 0.43 (Smith et al., 2016). A closer examination of Fig. 1 also reveals that the PIF matrix provides slightly greater thermal protection than CBF, as evidenced by the generally higher D_T values across a_w levels and temperatures. This is likely due to the composition of PIF, potentially containing more fat and protein than CBF, which are known to protect microorganisms during thermal treatments (Zhang et al., 2022). However, these differences could not be confirmed statistically.

The z values at the lowest a_w were considerably higher than those at highest a_w, indicating that increasing the intensity of thermal treatments is less effective in low moisture food compared with rehydrated products. Despite the relationship between the a_w and z values did not show a consistent linear trend for the different microorganisms and food matrix, the magnitude of the a_w effect was higher in *Salmonella* than in *P. acidilactici*. At low a_w in CBF, *Salmonella* exhibited greater thermal resistance to temperature changes (z = 26.38°C) compared to *P. acidilactici* (z = 13.88 °C). At a_w 0.75, *P. acidilactici* maintained its thermal resistance (z = 13.56 °C) and exceeded *Salmonella*, whose thermal resistance decreased (z = 11.71 °C) compared with that found in dried CBF (lowest a_w). In reconstituted CBF (a_w 0.98), *P. acidilactici* remained more resistant to temperature increase than *Salmonella*, with a difference of 2.29 °C. Concerning PIF, at a_w of 0.45 and 0.75, similar z values were observed for *P. acidilactici* (17.03 and 9.70 °C, respectively) and *Salmonella* (16.64 and 9.77 °C, respectively). However, at a_w of 0.98, z value for *P. acidilactici* was almost twice that of *Salmonella*.

The obtained results were consistent with Liu et al. (2018) and Wason et al. (2022) that also reported a significant reduction of z of *Salmonella* and *Enterococcus faecium* by increasing the a_w of the wheat flour and fine ground black pepper, respectively. However, the present study dealing with CBF and PIF, higher z value for *Salmonella* were observed. The differences in the estimated absolute z values compared to those reported in the literature may be attributed to biological variations among *Salmonella* serotypes and the matrix composition (e.g., fat and protein content), which can influence thermal resistance (Zhang et al., 2022).

3.2. Qualification of *P. acidilactici* as non-pathogenic surrogate of *Salmonella*

In order to understand the suitability of the strain *P. acidilactici* ATCC 8042 as a non-pathogenic surrogate of *Salmonella* for assessing the impact of PSD technology, the thermal inactivation parameters (D_T) obtained at relevant temperature conditions for PSD processing (e.g. at least 70 °C) were compared. The results of the linear regression analysis between LogD_T values of *P. acidilactici* and *Salmonella* across different food matrixes and a_w levels are shown in Supplementary Table 1. Overall, the regressions were highly significant ($p < 0.001$), with R^2 values ranging from 0.604 to 0.877, confirming the strong linear relationship between the thermal resistance of the two assessed microbial strains. The strongest correlation was found in PIF (R^2 = 0.877), suggesting more consistent thermal inactivation kinetics across conditions in this matrix compared to CBF (R^2 = 0.604). Similarly, a higher R^2 value was found for medium-high a_w (0.827) compared to low a_w (0.699). The significant slopes close to 1 (ranging from 0.987 to 1.223, $p < 0.001$) indicate a proportional relationship between the thermal resistance of the pathogen and the surrogate across the considered conditions (food matrix, a_w and thermal conditions). The intercepts, ranging from 0.503 to 0.871 (all significant, $p < 0.05$), reflect the higher resistance of the *P. acidilactici* over that of *Salmonella*.

The regression comparison revealed no statistically significant difference in regression slopes between food matrixes (CBF vs. PIF: p = 0.893) or between a_w levels (low vs. medium-high: p = 0.665), confirming that the overall relationship between *P. acidilactici* and *Salmonella* thermal responses remained stable regardless of environmental conditions. Therefore, an overall regression was performed as shown in Fig. 2. The clustering of points above and parallel to the equivalence line is indicative of the systematic higher thermal resistance exhibited by *P. acidilactici* compared to *Salmonella.* From a quantitative perspective, the calculated *Bf* was 4.42, which indicates that the decimal reduction time (D_T) of *P. acidilactici* strain was on average 4.42 times higher than that of the pathogen. Overall, data confirmed the robustness of *P. acidilactici* as a conservative (i.e. fail safe) surrogate for *Salmonella* suitable for in-plant thermal process validation procedures.

3.3. Survival of *P. acidilactici* during PSD processing

The strain *P. acidilactici* ATCC 8042 was used as a qualified non-pathogenic surrogate to assess the microbial inactivation during the drying of CBF and PIF by PSD technology. Fig. 3 shows the inactivation results recorded in the primary (main chambre) and secondary (cyclone) powders of CBF and PIF during PSD at different outlet temperatures. In CBF, microbial inactivation ranged 1.8 – 4.1 log units. At an outlet temperature of 95 °C, the secondary powder collected at 30 and 45 min showed less inactivation (1.9 ± 0.1 log) ($p < 0.05$) than the primary powder collected at 30 and 45 min (2.6 ± 0.1 log and 3.0 ± 0.2 log, respectively). The increase of the outlet temperature to 110 °C significantly increased the inactivation of *P. acidilactici* to an average of 3.5 ± 0.5 log reduction, with no significant differences between the type of powder and collection time.

The inactivation of *P. acidilactici* in PIF was comparable to that observed in CBF (Fig. 3B), ranging from 1.4 to 3.6 log reduction. Within each outlet temperature, no significant differences were observed between collection time or the type of powder, meaning that the sampling location (i.e. main chamber and cyclone) did not influence the bacterial survival. At an outlet temperature of 80 °C, the surrogate strain was inactivated by 1.8 ± 0.3 log. Increasing the outlet temperature to 90 °C led to a significant increase in the inactivation (2.8 ± 0.3 log), that was no further enhanced at 100 °C (3.0 ± 0.3 log).

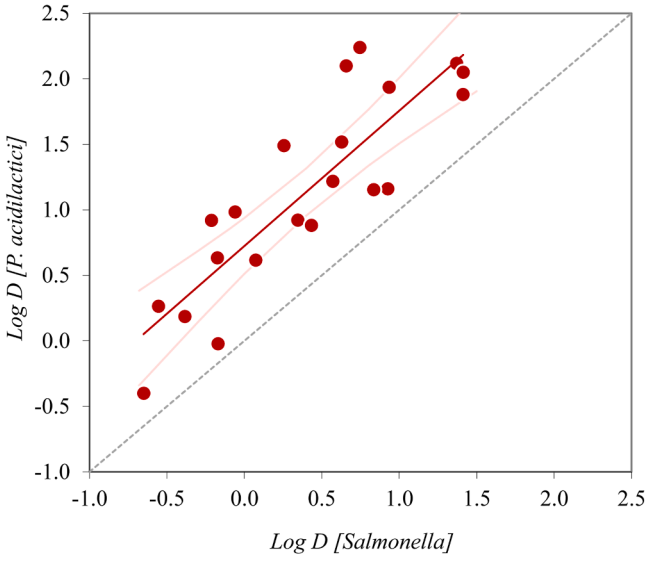

Fig. 2. Comparison of log-transformed D_T values between *P. acidilactici* ATCC 8042 and *Salmonella enteritidis* serovar Senftenberg ATCC 43845 in infant food matrixes (including cereal-based food and powdered infant formula with different a_w). Each dot represents a pair of D_T values under identical processing temperature. The red solid line indicates the linear regression fit (95% confidence interval shown in light red, see statistics in Supplementary Table 1), while the grey dashed line corresponds to the 1:1 equivalence line.

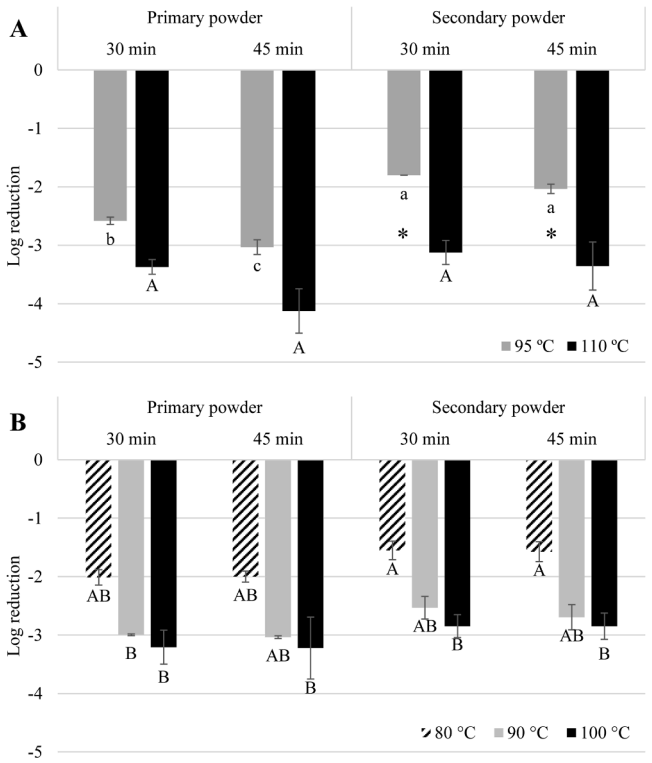

Fig. 3. Inactivation of *P. acidilactici* ATCC 8042 in infant food by Pulse Spry Drying (PSD), sampled in the main chambre (primary powder) and cyclone (secondary powder) at 30 and 45 min of the drying process: (A) in cereal-based food (CBF) at PSD outlet temperature of 95 and 110 °C. Different lowercase letters indicate significant differences between CBF samples at 95 °C; different capital letters indicate significant differences between CBF samples at 110 °C. Asterisk (*) indicates significant increase between outlet temperatures in CBF samples. (B): in powder infant formula (PIF) at PSD outlet temperature 80, 90 and 100 °C. Different capital letters indicate significant differences ($p < 0.05$).

In concordance with these results, Dantas et al. (2024) also reported no influence of the sampling location and collection time on the *P. acidilactici* survival in skimmed milk dried by PSD at outlet temperatures of 80, 95 and 110 °C. In addition, Steinbrunner et al. (2021) reported that the place of sampling (main chamber or cyclone) did not significantly impact on bacterial reduction in soy protein isolate dried using pilot-scale spray drying. By contrast, Arku et al. (2008), in a lab-scale spray drying of reconstituted skim milk at an outlet temperature of 90 °C, found that the influence of sample collection time on the microbial inactivation depended on the bacterial strains of *Cronobacter sakazakii*.

Microbial inactivation during the drying process is mainly promoted by the high temperature, which is known to induce denaturalization of key cellular components and the interruption of normal metabolism and division (Gong et al. 2014). Even if microbial inactivation depends on drying parameters such as inlet/outlet temperatures, residence time and feed rate, the outlet temperature at which the product leaves the drying chamber is believed to be the major drying parameter affecting viability of microorganisms (Santivarangkna et al., 2008). Moreover, microbial inactivation is also produced through dehydration, which cause osmotic imbalance (cytoplasm changes) and damage to cellular structures (Ananta et al., 2005; Bhandari and Howes, 1999; Gong et al., 2014).

In the present study, the increase of the outlet temperature slightly reduced the viability of *P. acidilactici*, being significant only in some cases. Dantas et al. (2024) also observed that the outlet temperatures significantly influenced *P. acidilactici* inactivation in skimmed milk dried by PSD at 80, 95 and 110 °C. Contrary, Steinbrunner et al. (2021) did not find significant effect of inlet temperature (180, 200 and 220 °C) on the microbial inactivation of *Salmonella* and *Enterococcus faecium* in soy protein isolate dried by spray drying.

3.4. Formation of process-induced contaminants during PSD

HMF is formed as an intermediate in the Maillard reaction, through decomposition of 3-deoxyglucosone, or by caramelization, through dehydration of sugars (Capuano and Fogliano, 2011). No HMF was detected in reconstituted CBF and PIF used to feed the PSD equipment. Fig. 4 shows the HMF content in CBF after PSD drying process. HMF content increased slightly with increasing outlet temperature (95 and 110 °C). At 95 °C of outlet temperature, no significant differences were observed in HMF content between powder types and sampling times (0.15 ± 0.02 mg/kg). While at 110 °C of outlet temperature, primary powder collected at 45 min (0.34 ± 0.04 mg/kg) showed more formation of HMF compared to the other samples. The observed significant increase may be at least partially attributed to the primary powder adhering to the wall of the main chamber being exposed longer to high temperature. The extended thermal exposure likely promoted the formation of HMF, as time and temperature are processing conditions directly correlated with the generation of this compound (Gökmen and Morales, 2014). In PIF, HMF did not show significant differences between outlet temperature (80, 90 and 100 °C), powder types or collection time of PSD, with a mean content of 0.14 ± 0.07 mg/kg. Even if low levels of HMF were detected in PIF after PSD, the thermal conditions were likely too mild to induce significant changes among the assessed outlet temperatures, powder types and collection times.

Currently, there are no established limits for the HMF content in powdered infant foods. In honey, a maximum level of 40 mg/kg is set, except honey produced in tropical climates (80 mg/kg), and honey with low enzymatic activity (15 mg/kg) (FAO/WHO, 2019). This lack of regulation complicates the assessment of whether the HMF levels found in the studied foods are acceptable or excessive. The HMF content observed in the present study was comparable with other studies. Regarding CBF, 0.71 mg/kg of HMF were reported in commercial rice-based infant food (Ramírez-Jiménez et al., 2003), 1.64 – 16.60 mg/kg in wheat, rice and oat-based infant food (Fernández-Artigas et al., 1999) and 0.57 – 13.5 mg/kg in rice, rice-corn and multi cereal-based infant food (Solís-Casanova et al., 2011). Regarding PIF, Cui et al. (2020) found HMF levels between 0.06 to 0.37 mg/kg. In addition, Vella & Attard (2019) found a range of 0.29 to 7.87 mg/kg of HMF in commercial infant formula produced by conventional spray drying. Therefore, PIF samples with PSD technology showed HMF levels within the lower bound of the range reported in several commercial infant food dried using conventional drying technologies.

On the other hand, acrylamide is also formed by Maillard reaction

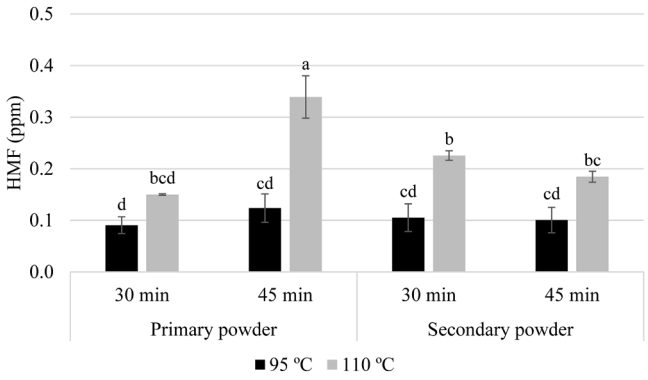

Fig. 4. Hydroxymethylfurfural (HMF) concentration in primary and secondary powders of cereal-based infant food (CBF) dried by PSD at different outlet temperatures (95 °C and 110 °C) and collected at 30 and 45 min of the drying process. Different letters indicate significant differences ($p < 0.05$) between powder samples.

with free asparagine and carbonyl sources as the main precursors. However, key factors contributing to acrylamide formation in food are processing temperatures above 120 °C, low moisture product, and an inactive matrix such as starch (Mojska et al., 2012). Thus, acrylamide was considered in CBF given its industrial processing conditions, low moisture and starch content. In this regard, CBF showed 119.5 ± 34.3 μg/kg of acrylamide, with no significant differences between outlet temperatures (95 and 110 °C), powder types or collection times of PSD. Similar contents were reported by Mojska et al. (2012) that found 148 ± 54 μg/kg of acrylamide in powdered commercial infant cereals from Poland in the 2007–2009 period. These levels are below the benchmark levels for biscuits and rusks for infants (150 μg/kg) but exceeds the benchmark level for infant food (processed cereal-based foods, 40 μg/kg) set by Commission Regulation (EU) 2017/2158 (European Commission, 2017). Therefore, further studies should be carried out to find the suitable PSD drying process conditions to reduce the level of acrylamide in CBF and maintain the characteristics of the final product.

4. Conclusions

This study provides the first evidence supporting the use of the non-pathogenic *P. acidilactici* strain ATCC 8042 as a suitable conservative surrogate of *Salmonella* for the assessment of the impact of a thermal-based drying technology applied to infant food, including cereal-based food (CBF) and powdered infant formula (PIF). This contribution addresses a gap in food safety, enabling safer and more practical validation of emerging technologies at pilot plant scale. Our findings demonstrate that PSD, which is an innovative drying technology for drying infant food (CBF and PIF), can achieve significant microbial inactivation, with efficacy dependent on the processing conditions.. Importantly, the formation of process-induced contaminants such as HMF and acrylamide, associated with the PSD process, remained within ranges reported for commercial products, underscoring the potential of PSD to balance microbial safety and chemical quality. These results position PSD as a promising alternative to conventional drying methods, offering a viable solution for enhancing the safety and quality of infant foods while supporting food industry innovation and regulatory compliance.

CRediT authorship contribution statement

Berta Torrents-Masoliver: Writing – original draft, Methodology, Investigation, Formal analysis. **Emmanuelle Boix:** Writing – original draft, Methodology, Investigation. **Gisela Quinteros:** Writing – original draft, Methodology, Investigation. **Anna Jofré:** Writing – review & editing, Supervision, Investigation, Data curation, Conceptualization. **Albert Ribas-Agustí:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Xavier Felipe:** Writing – review & editing, Methodology, Conceptualization. **Sara Bover-Cid:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Statement

No studies in humans and animals

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100745.

Data availability

Data will be made available on request.

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