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Modelling the survival of *Listeria monocytogenes* in oat plant-based milk alternatives: The synergistic potential of HHP and nisin

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ABSTRACT

The growing popularity of plant-based milk alternatives (PBMAs), particularly oat-based beverages, is driven by consumer demand for sustainable, vegan, and lactose-free options. High Hydrostatic Pressure (HHP) is a non-thermal technology capable of extending shelf life and enhancing microbial safety, especially when combined with natural antimicrobials such as nisin. This study aimed to quantitatively assess the inactivation of *L. monocytogenes* strains LO28 and 10403S in oat-based PBMA treated with HHP alone or in combination with nisin (500 IU/mL). Inoculated oat PBMA samples with or without nisin were subjected to 300, 350, and 400 MPa (20 °C) for different holding times, up to 40 min. Inactivation followed a log-linear behavior at 300 MPa and biphasic kinetics at 350 and 400 MPa. The addition of nisin significantly ($p < 0.05$) reduced the time to 3-log reduction ($3D_P$) compared to HHP alone at all pressures for LO28 and at 400 MPa for 10403S. For LO28 at 350 MPa, $3D_P$ values decreased from 7.65 ± 0.42 min (HHP alone) to 3.11 ± 0.28 min (HHP + nisin), indicating a synergistic effect as nisin alone had no impact. Secondary modeling confirmed that HHP efficacy was both strain- and nisin-dependent. Pressure resistance (z_P) was significantly lower for LO28 (102.7 ± 2.7 MPa) than 10403S (193.8 ± 10.6 MPa). Nisin notably reduced z_P in 10403S (193.8 ± 10.6 to 107.4 ± 4.6 MPa) enhancing its pressure sensitivity. These findings demonstrate that HHP combined with nisin represents an effective hurdle strategy for producing safe, refrigerated, clean-label oat-based PBMAs. Optimized processing conditions may allow lower pressures or shorter treatment times, reducing costs while preserving product quality. Further work should assess microbial stability during refrigerated storage and consumer acceptance.

1. Introduction

The demand for plant-based milk alternatives (PBMAs) has emerged in recent years widening the range of options available to consumers, particularly those following vegan or lactose-free diets (Giugliano et al., 2023). Among the various PBMAs, oat-based milk alternatives have gained significant attention due to their unique nutritional profile and favorable sensory attributes. In 2020, the oat PBMA market size was valued at $2.23 billion globally and is expected to grow at a compound annual growth rate (CAGR) of 14.2 % from 2020 to 2028. While North America remains a key market for PBMAs, the Asia-Pacific region has emerged as the largest consumer and fastest-growing market in this sector (Grand View Research, 2025). This popularity can be largely

attributed to the presence of β-glucan, a highly soluble fiber with recognized health-promoting properties. Research indicates that β-glucan has the potential to lower blood cholesterol levels, thereby reducing the risk of coronary heart disease as recognized by EFSA (EFSA Panel on Dietetic Products Nutrition and Allergies, 2010). Additionally, it has been associated with anticancer properties, further enhancing the health benefits of oat beverages (Aktas-Akyildiz et al., 2018; Cui et al., 2023).

Listeria monocytogenes is a ubiquitous and persistent foodborne pathogen capable of surviving in challenging environmental conditions, posing a significant risk to food safety and public health. Numerous studies have shown that *L. monocytogenes* can grow in plant-based beverages, making it a notable microbial hazard within this food

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category (Bartula et al., 2023; Kain et al., 2024; Azizoglu, 2024). These beverages show favorable pH and water activity and contain sufficient nutrients to support bacterial growth, even in the absence of added sugars, proteins, or fats, particularly when appropriate preservation strategies are not implemented (Bartula et al., 2024). Outbreaks of listeriosis have been linked to plant-based beverages, including oat PBMA, with the most recent and significant case occurring in Canada in July 2024, resulting in three deaths and 15 hospitalizations due to severe infections (Public Health Agency of Canada, 2024). Ultra-High-Temperature (UHT) processing is commonly applied to oat-based beverages to ensure microbial stability and extend shelf-life for several months at ambient temperatures. However, this processing technique adversely affects flavor, nutrients and other heat sensitive compounds (Reyes-Jurado et al., 2023). For instance, β-glucan content was found to decrease significantly following initial heat treatment at 60 °C, highlighting the detrimental effect of thermal processing on the nutritional quality of oat-based dairy alternatives (McCarron et al., 2025).

In recent years, non-thermal pasteurization techniques, such as High Hydrostatic Pressure (HHP), have gained attention for their ability to preserve fresh-like product characteristics while improving consumer acceptance. In line with this, HHP has been shown to better preserve fresh-like aroma and flavor in fruit beverages compared with thermal treatment (Song et al., 2022). HHP in the range of 200–600 MPa has been shown to effectively inactivate pathogenic and spoilage microorganisms, without the adverse effects associated with conventional heat pasteurization (Aganovic et al., 2021; Kim et al., 2018; Wang et al., 2016). More specifically, Ahmad et al. (2025) reported that HHP at appropriate pressure levels can improve the viscosity, color, protein content, and homogenization of oat PBMA compared to heat treatment, resulting in desirable rheological and sensory properties while maintaining or enhancing nutritional quality. Recent work by Zhang et al. (2022) has shown that HHP at 400 MPa can enhance aroma formation in soy sauce by promoting Maillard, oxidation, and hydrolysis reactions, leading to a sensory profile comparable to long-term fermentation. Despite these benefits, the impact of HHP on pathogenic bacteria in plant-based dairy alternatives remains underexplored, particularly with respect to *L. monocytogenes* in oat PBMAs.

Research has shown that combining HHP with antimicrobial agents such as bacteriocins, chitosan, lysozyme, and lactoferrin enhances antimicrobial efficacy compared to HHP treatment alone in laboratory media and various food matrices (Bigi et al., 2023; Giannoulis & Karatzas, 2024; Hereu et al., 2012; Yang et al., 2021). This hurdle technology approach offers several advantages, including reduced energy consumption, improved microbial safety, and opportunities to expand the product portfolio, particularly in the growing sector of plant-based alternatives. Nisin, an antimicrobial peptide naturally produced by *Lactococcus lactis*, has attracted considerable interest as a natural food preservative. It is among the few natural antimicrobials approved for direct use in specific food products, as authorized by Commission Regulation (EU) No 1129/2011 (European Commission, 2011) and has a GRAS (Generally Recognized As Safe) status by the U.S. Food and Drug Administration, (2001) (GRAS Notice ID: 65). It has been well-studied that nisin possesses antimicrobial properties against various Gram-positive bacteria, effectively enhancing food safety and extending shelf life (Setiarto et al., 2023). It has also been shown to possess notable antilisterial activity across various food matrices including fresh, pasteurized, and homogenized milk, as well as juices and plant-based beverages (Gharsallaoui et al., 2016; Wu et al., 2023). According to Bhatti et al. (2004), nisin (250 IU/mL) shows strong antilisterial activity across various milk types, with the highest effectiveness observed in skim and 2 % fat milk, where *L. monocytogenes* levels (inoculated at 10^4 CFU/mL) were reduced to below 10 CFU/mL after 12 days at 5 °C. Furthermore, nisin exhibited significant antimicrobial effect during the fermentation of carrot juice and soy-based milk alternative, lowering *L. monocytogenes* counts from 10^4 CFU/mL to below the detection limit

(Siroli et al., 2019).

The combination of HHP and nisin has been found to exert a synergistic antimicrobial effect in a range of food products (Giannoulis et al., 2025; Martillanes et al., 2021) including dairy matrices such as skim milk. Black et al. (2005) reported an 8-log reduction of *L. innocua* in milk treated with 500 MPa for 5 min at 20 °C in the presence of 500 IU/mL nisin. This synergy has also been demonstrated in fruit juices; Pokhrel et al. (2019) reported a significant reduction of *L. monocytogenes* in carrot juice using a combination of HHP (400 MPa, 2 min) and nisin. However, to the best of the author's knowledge, no previous studies have investigated the effect of HHP alone or in combination with nisin against *L. monocytogenes* in oat-based milk alternatives.

The objective of this study was to quantitatively characterize the effect of HHP alone or combined with nisin on the inactivation of *L. monocytogenes* LO28 and 10403S strains in an oat-based milk alternative. The overall aim was to use this knowledge to optimize HHP processing conditions and support the safe production of refrigerated, clean-label, minimally processed oat-based PBMA.

2. Materials and methods

2.1. L. monocytogenes strains and inoculum preparation

L. monocytogenes strains LO28 (serotype: 1/2c; origin: healthy pregnant carrier) and 10403S (serotype: 1/2a; origin: human skin lesion) available at the Department of Food & Nutritional Sciences (University of Reading) strain collection were used in this study. These strains were selected due to their extensive characterization and widespread use as reference strains in *L. monocytogenes* research dealing with HHP (Tsagkaropoulou & Karatzas, 2024; Van Boeijen et al., 2008). Frozen stock cultures were prepared in 7 % v/v dimethyl sulfoxide (DMSO; Sigma Aldrich, Dorset, UK) and stored at −80 °C. For refrigerated storage (4–5 °C), frozen cultures were streaked onto BHI agar plates (BHI agar; NCM0080A, Neogen, Lancashire, UK). After overnight incubation at 37 °C, the plates were stored at refrigeration temperature and refreshed monthly with newly streaked cultures. Prior to each experiment, both strains were revived by selecting three isolated colonies from the agar plate and inoculating them into 3 mL of BHI broth (BHI; NCM0016A, Neogen, Lancashire, UK) in sterile plastic tubes (ThermoFisher, UK). The cultures were incubated at 37 °C for 24 h with shaking at 120 rpm. Subsequently, 10 mL of BHI broth in sterile Falcon tubes (ThermoFisher, UK) was inoculated with 1 % (v/v) of the primary culture and incubated at 37 °C for 16–18 h with shaking (120 rpm) until stationary phase was reached.

2.2. Oat PBMA sample preparation and nisin application

Commercially available Ultra-High Temperature (UHT) processed oat PBMA was used as the tested matrix in this study to avoid interference of background microbiota. The product contained water, 10.5 % w/w oat, soluble corn fibre, and sunflower oil, along with added minerals (tri-calcium phosphate, potassium iodide), vitamins (B2, D2), sea salt, and a stabiliser (gellan gum). The nutritional information is summarized in Supplementary Table, ST1. The pH of the oat PBMA was 7.10 ± 0.02, as measured using a pH meter (Mettler Toledo, UK). Stationary-phase cultures of both *L. monocytogenes* strains (LO28 and 10403S) were harvested by centrifugation at 1916×*g* for 10 min at 4 °C in a 5804 R Eppendorf centrifuge. The resulting cell pellets were resuspended in the oat PBMA, yielding a cell suspension with an initial target concentration of approximately 10^9 CFU/mL.

Nisin was applied in the form of the commercial preparation Nisaplin® (containing 2.5 % nisin (E234) corresponding to 1000 IU/mg; distributed by International Flavors & Fragrances, Inc.). A nisin stock solution (5000 IU/mL; 0.125 mg/mL) was freshly prepared prior to each experiment by dissolving 0.05 g of Nisaplin® in 10 mL of oat PBMA in a sterile Falcon tube. An appropriate aliquot of the stock solution was

subsequently added to the inoculated oat PBMA to achieve a final nisin concentration of 500 IU/mL (0.0125 mg/mL). This concentration level corresponds to the maximum accepted in dairy products by the FAO/WHO Codex Committee on Milk and Milk Products (Codex Alimentarius Commission, 2011) and is the maximum permitted in European Union under Commission Regulation (EU) No 1129/2011 (EU, 2011).

2.3. HHP equipment

HHP treatments were carried out using a lab-scale HHP equipment (Stansted Fluid Power Products Ltd., Harlow, UK), which featured a vessel with a 1.8 cm internal diameter and a 29 mL working volume (Fig. 1) The pressure come-up-time ranged from 0.9 to 1.3 min depending on the pressure level (Suppl. Table, ST4), while decompression occurred almost instantaneously (within 2–3 s). The main parameters of each treatment were the pressure (MPa) and the holding time (min) while "Time 0" (or "Pulse treatment") was considered as the exposure to pressure during the come-up time only, with no additional holding time. The temperature of the HHP chamber was monitored throughout each treatment using a digital thermometer (PCE-T 390, PCE Instruments, Southampton, UK) connected to a K-type thermocouple placed in direct contact with the pressure-transmitting liquid (20 % v/v castor oil in ethanol).

2.4. Experimental design

A fixed volume of 1 mL of the oat PBMA either with nisin (**HHP + nisin**) or without (**HHP alone**) and inoculated with one of the *L. monocytogenes* strains (LO28 or 10403S), was transferred into sterile plastic stomacher bags (Seward, London, UK) with dimensions of 3.5 cm × 4 cm. To prevent cross-contamination, the bags were double-sealed prior to pressurization. The HHP inactivation was investigated in an oat PBMA at three pressure levels (300, 350 and 400 MPa) at 20 °C for defined holding times up to 40 min. The selected pressure range was based on the pressure resistance of the strains and was spaced at 50 MPa intervals, while also considering the maximum operational limit of the HHP system (400 MPa). The range of holding times was chosen to ensure

Fig. 1. Schematic diagram of the laboratory-scale HHP system used in this study.

that sufficient time points were obtained to describe the inactivation kinetics, particularly at the lower pressures. This approach allowed the appropriate primary model to be fitted to the inactivation data for each pressure level and treatment condition (with or without nisin; Suppl. Table, ST4). Similar pressure levels were applied to both strains under both conditions to allow for direct comparison of the resulting inactivation kinetics and the estimation of the decimal reduction time (D_P value), defined as the pressure-holding time (min) necessary to inactivate the microbial population by 1 log (90 % reduction). Each experiment was performed in three biologically independent replicates, and each replicate produced an individual inactivation curve, resulting in a total of 36 curves, with primary models fitted to each curve separately.

2.5. Enumeration of viable cells

Following pressurization, HHP-treated samples were immediately opened under aseptic conditions and serially diluted in Maximum Recovery Diluent (MRD; Fisher Scientific, UK). Appropriate dilutions were spread-plated onto BHI agar. In addition, the initial concentration of *L. monocytogenes* in oat PBMA, with or without nisin, was enumerated prior to HHP treatment to confirm the inoculum concentration (Suppl. Table, ST2). Moreover, samples with added nisin but without HHP treatment were also enumerated at the end of each experiment to assess the effect of nisin alone on *L. monocytogenes* cells (Suppl. Table, ST3). All plates were incubated at 37 °C for 48 h. The lower detection limit of this enumeration method was 2.1 log CFU/mL.

2.6. Model fitting

Primary models (Table 1) were fitted to the survival data (log CFU/mL vs time) obtained from HHP alone and in combination with nisin, for each pressure level between 300 and 400 MPa and for each replicate. The models were fitted using the GInaFiT add-in freeware tool for Microsoft Excel (Geeraerd et al., 2005). Model performance was evaluated using goodness-of-fit metrics, including the residual sum of squares (RSS), root mean squared error (RMSE), and the coefficient of determination (R^2). To identify the simplest and best-fitted model, pairwise comparisons were conducted using an F-test, following the methodology outlined by Van Boeijen et al. (2008).

The estimated kinetic parameters of the best fitting primary model were used to calculate the time required to achieve a 3-log reduction. The HHP time needed to achieve 3-log reduction, i.e. $Log(3D_P)$ was calculated for the log-linear behavior (Eq. (5); Patil et al., 2010) as well as the biphasic behavior (Eq. (6); Torrents-Masoliver, Serra-Castelló, et al., 2025) when relevant.

$$\log(3D_P) = \log\left(\frac{3}{k_{max}/\mathrm{Ln}(10)}\right) \qquad \text{Eq. (5)}$$

Table 1
Primary inactivation models used to fit microbial HHP survival data.

Model	Equation[a]	Reference	
Log-linear	$\log(N) = \log(N_0) - kmax \cdot t/Ln(10) = \log(N_0) - t/D$	Bigelow and Esty (1920)	Eq. 1
Log-linear with tail	$\log(N) = \log(10^{\log(N_0)} - 10^{\log(N_{res})}) \cdot e^{-kmax \cdot t} + 10^{\log(N_{res})})$	Geeraerd et al. (2000)	Eq. 2
Weibull	$\log(N) = \log(N_0) - (t/\delta)^p$	Mafart et al. (2002)	Eq. 3
Biphasic	$\log(N) = \log(N_0) + \log(f \cdot e^{-kmax_1 \cdot t} + (1-f) \cdot e^{-kmax_2 \cdot t}$	Cerf (1977)	Eq. 4

[a] Log (N): microbial counts after specific time (t), log (N_0): initial microbial counts, $kmax$: inactivation rate (1/time); *t*: time; log N_{res}: maximum inactivation, tail; δ: time for the first log reduction; *p*: shape of the inactivation curve, *f*: fraction of initially major subpopulation least resistant.
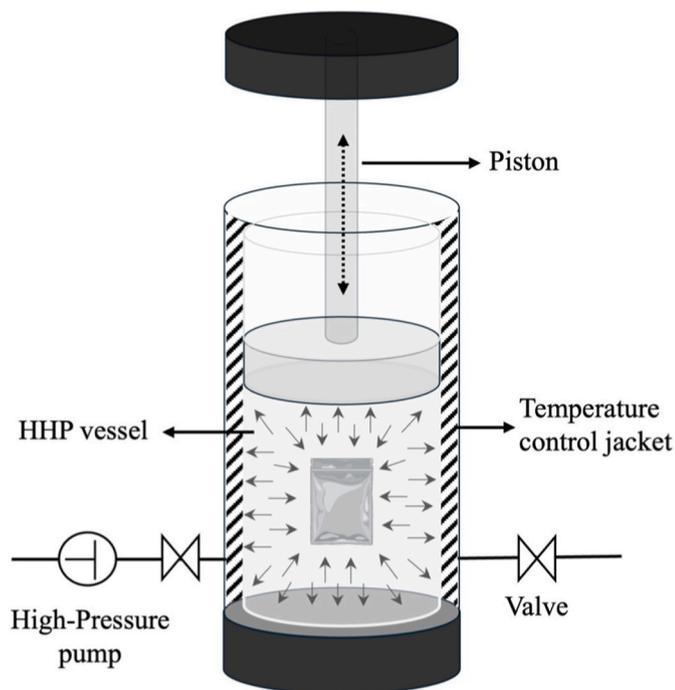
If $f^* \geq 3$; $\log(3D_P) = \log\left(\frac{3}{k_{max\ 1}/\mathrm{Ln}(10)}\right)$

If $f^* < 3$; $\log(3D_P) = \log\left[\left(\frac{f^*}{k_{max\ 1}/\mathrm{Ln}(10)}\right) + \left(\frac{3-f^*}{k_{max\ 2}/\mathrm{Ln}(10)}\right)\right]$

Being $f^* = -\log(1-f)$ Eq. (6)

Where, k_{max} is the inactivation rate (Ln/min) in the log-linear phase (estimated from Eq. 1; Table 1); k_{max1} and k_{max2} are the inactivation rates (Ln/min) in each log-linear phase obtained in the biphasic model corresponding to the sensitive and resistant subpopulations to HPP, respectively; f is the cell concentration (log) corresponding to the fraction of the initially major subpopulation that is least resistant to HHP estimated with Eq. (4) and f^* is the log concentration of the fraction of the initially major subpopulation that is resistant to HHP.

The impact of pressure and nisin (500 IU/mL) on estimated three times decimal reduction values at each pressure ($3D_P$) was described by the Bigelow-type secondary model (Eq. (7)). Secondary model fitting was carried out with the MS Excel Solver Add-in.

$$\log 3 D_P = \log 3 D_{Pref} + \frac{P_{ref} - P}{z_P}$$ Eq. (7)

Where, $\log 3D_{Pref}$ is the logarithm of the time (min) to achieve three log reductions at a reference pressure ($P_{ref} = 400$ MPa) and z_P represents the increase of the pressure (P in MPa) necessary to reduce the $\log 3D_P$ by a factor of 10.

2.7. Statistical comparisons

To evaluate the effect of HHP alone and in combination with nisin, as well as to compare the responses of the two strains and assess treatment efficacy, z_P values obtained from secondary modeling (at a reference pressure of 400 MPa) were statistically compared using one-way ANOVA, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. A probability value of <0.05 (*p-value* < 0.05) was considered statistically significant for all comparisons. All statistical analyses were carried out using GraphPad Prism (version 10.2.2).

3. Results and discussion

3.1. Inactivation kinetics, primary model fitting

Figs. 2 and 3 show the inactivation kinetics of *L. monocytogenes* strains LO28 and 10403S in an oat-based milk alternative subjected to HHP at 300, 350 and 400 MPa for up to 40 min, with and without the addition of nisin (500 IU/mL), across three biological replicates. At 300 MPa, inactivation of both strains by HHP alone showed a linear reduction in log of viable cell counts over pressure holding time, indicating first-order kinetics. Consequently, the log-linear model was the most appropriate for describing the inactivation trend at this pressure. A log-linear relationship between *L. monocytogenes* survivors and treatment time has been reported in various liquid food products including milk, orange and peach juices, subjected to HHP (Dogan & Erkmen, 2004; Huang et al., 2015; Kateh et al., 2024; Possas et al., 2017). At pressures above 300 MPa, both *L. monocytogenes* strains showed non-linear inactivation behavior. The two-parameter Weibull model (δ and p) is the most popular applied model to describe such non-linear microbial inactivation under HHP, due to its mathematical flexibility and simplicity (Serment-Moreno et al., 2017; Usaga et al., 2021). In the present study, however, the biphasic model (Cerf, 1977) provided a better fit to the experimental data, as indicated by RSS and RMSE values. This biphasic behavior is likely due to the presence of two subpopulations with differing pressure resistance. The natural occurrence of piezotolerant subpopulations, potentially arising from naturally occurring mutants, has previously been described in *L. monocytogenes* and *S. aureus* and, in some cases, has been associated with hypermutable

Fig. 2. Inactivation kinetics of *L. monocytogenes* LO28 in an oat PBMA subjected to HHP at 300, 350, and 400 MPa, with ($\triangle$ – HHP + nisin) or without ($\blacktriangle$ – HHP alone) the addition of nisin (500 IU/mL), across three independent biological replicates. Cell counts (log CFU/mL) are plotted against HHP treatment time (min) for each replicate. Triangles represent observed experimental values, and the lines correspond to the fitted primary inactivation model.
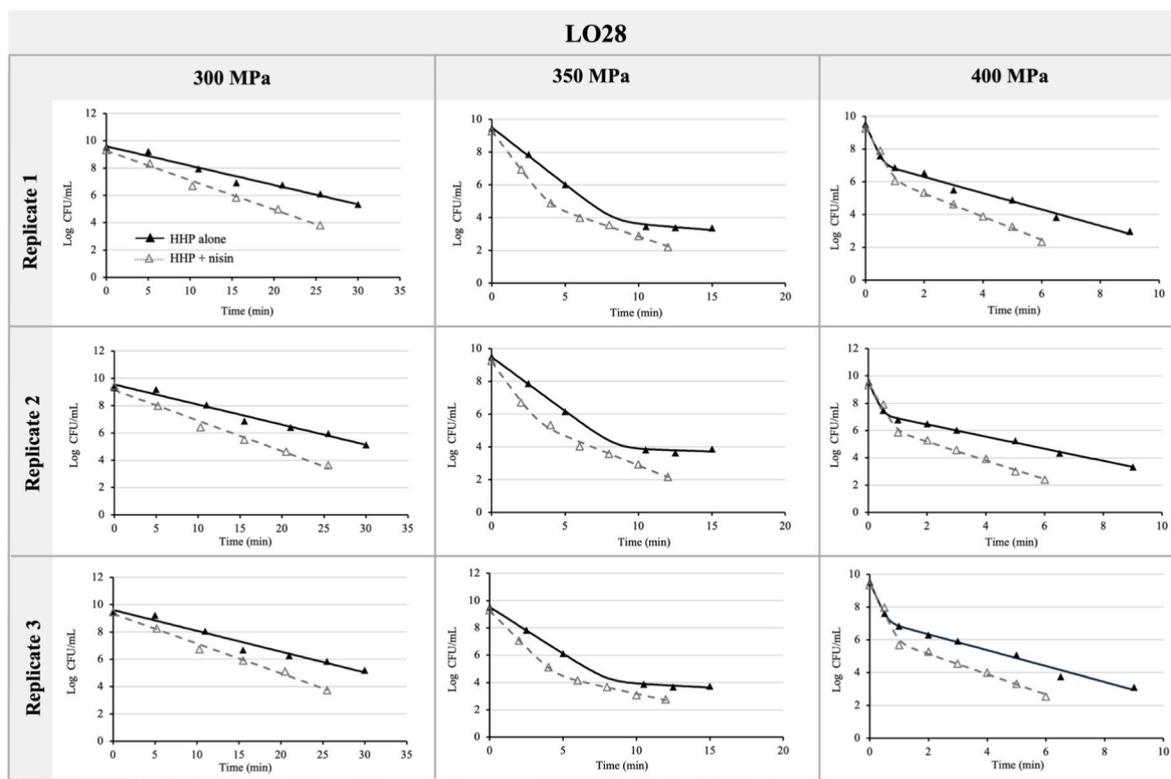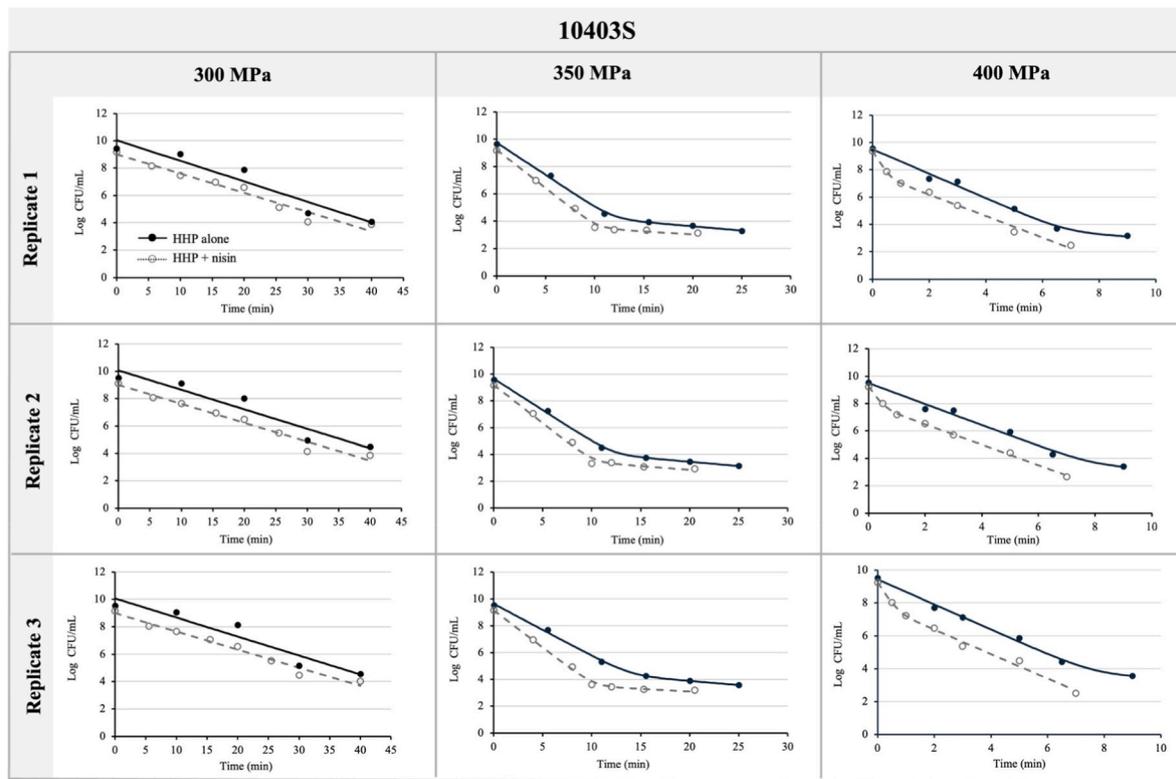
Fig. 3. Inactivation kinetics of *L. monocytogenes* 10403S in an oat PBMA subjected to HHP at 300, 350, and 400 MPa, with (○ – HHP + nisin) or without (● – HHP alone) the addition of nisin (500 IU/mL), across three independent biological replicates. Cell counts (log CFU/mL) are plotted against treatment time (min) for each replicate. Circles represent observed experimental values, and the lines correspond to the fitted primary inactivation model.

regions in stress gene regulators such as *ctsR* (Karatzas & Bennik, 2002; Karatzas et al., 2003). This biphasic behavior has also been used to describe the inactivation of *L. monocytogenes* and *Salmonella enterica* in milk during HHP treatment (Van Boeijen et al., 2008; Xu et al., 2009). The biphasic model has also been applied to describe the inactivation of naturally occurring microbiota in cucumber juice subjected to 300–400 MPa (Zhao et al., 2014). Similarly, Lee et al. (2001) reported that *E. coli* inactivation in liquid whole egg under HHP (250–400 MPa) showed a biphasic behavior due to tailing in the survival curves.

The addition of nisin (open symbols with dashed grey lines; Figs. 2 and 3) to the oat PBMA did not affect the selection of the primary model to describe microbial inactivation kinetics during HHP treatment for both strains. As with HHP alone, the log-linear model was selected at 300 MPa, while the biphasic model provided the best fit at 350 and 400 MPa. However, at 400 MPa, the biphasic behavior became more evident when HHP was combined with nisin, particularly for the 10403S strain. This is consistent with findings by Serra-Castello et al. (2025), who reported that different cooked ham formulations (e.g., sodium-reduced formulation) and packaging conditions did not affect the selection of inactivation kinetic model (Weibull) for *L. monocytogenes* during HHP processing. The initial inactivation rate (k_{max1}) significantly increased from 1.7 to 2.1 min^{-1} with HHP alone to 6.2–7.7 min^{-1} with HHP + nisin treatment, indicating a more rapid decline in viable cell counts in the early stages of the treatment. The estimated parameters (k_{max1}, k_{max2}, f, RMSE, RSS, R_{adj}^2) of the inactivation kinetics for each data set are presented in Supplementary Tables 6 and 7

HHP Come-Up-Time (CUT), defined as the pressure increase phase without a holding time (i.e., pulse treatment), has occasionally been reported to cause microbial inactivation depending on the pressure level (Torrents-Masoliver, Jofré, et al., 2025). However, in the present study, no inactivation of either *L. monocytogenes* strain was observed at any pressure level during CUT, regardless of the presence of nisin (Suppl. Table, ST5). This finding aligns with previous research, where only

minor reductions in *L. innocua* counts (0.2–0.7 log) were reported in milk subjected to CUT at 350–600 MPa (Serment-Moreno et al., 2017).

For comparative purposes on the impact of the studied factors, the time (min) required to achieve a 3-log reduction ($3D_P$) of both *L. monocytogenes* strains, with or without nisin, in the oat PBMA was calculated using Eqs. (5) and (6) (Table 2). The $3D_P$ parameter was selected in this study as a reliable kinetic indicator for comparative analysis. This approach avoids extrapolating beyond the experimental data range and prevents bias due to curve shape, as recommended by Guillou and Membré (2019) and Valdramidis et al. (2005). Moreover, it allows an accurate and direct comparison between the two different

Table 2
Estimated $3D_P$ values (min) for *L. monocytogenes* strains LO28 and 10403S in oat-based PBMA subjected to HHP at 300, 350, and 400 MPa, with and without the addition of nisin (500 IU/mL).

Condition	LO28	10403S
	$3D_P$ (min)	$3D_P$ (min)
300 MPa	[a]20.4 ± 0.75	[a]20.90 ± 0.89[ns]
300 MPa + nisin	[b]13.7 ± 0.07	[a]21.89 ± 0.71*
350 MPa	[c]7.65 ± 0.42	[b]10.91 ± 0.83*
350 MPa + nisin	[d]3.11 ± 0.28	[b]9.42 ± 0.20*
400 MPa	[d]2.16 ± 0.07	[c]6.37 ± 0.55*
400 MPa + nisin	[e]1.00 ± 0.22	[d]2.57 ± 0.28*

$3D_P$ value represents the estimated treatment time required to achieve a 3-log reduction in cell counts.

Values are presented as means ± standard deviation (n = 3 biological replicates).

[a-d]: Different superscript letters within the same strain indicate statistically significant differences between conditions (Tukey's HSD test; $p < 0.05$).

(*) Asterisk indicates statistically significant differences in $3D_P$ values between the two strains while "ns" denotes no significant difference (*t*-test, $p < 0.05$).

strains and treatments.

Across most of the pressure levels tested, the addition of nisin resulted in lower $3D_P$ values compared to HHP alone. More specifically, for LO28 strain, the combination of HHP and nisin significantly reduced the time required for a 3-log reduction at all pressure levels ($p < 0.05$). For instance, at 350 MPa, the $3D_P$ value decreased from 7.65 ± 0.42 min (HHP alone) to 3.11 ± 0.28 min with the addition of nisin (Table 2). Considering that nisin alone did not reduce *L. monocytogenes* counts in both strains throughout the experiment (Suppl. Table, ST3), the observed decrease in $3D_P$ values could be attributed primarily to the combined effect of HHP and nisin. These findings suggest a potential synergistic effect where HHP, which is known to disrupt cell membranes and structural integrity, may enhance the antimicrobial activity of nisin, which acts by forming pores in the cytoplasmic membrane and inhibiting peptidoglycan biosynthesis (Simons et al., 2020). Thus, targeting of the same molecular structures by both stresses may lead to extensive damage or disruption, thereby enhancing their combined antimicrobial efficacy. The synergistic effect of HHP and nisin (or other bacteriocins) against *L. monocytogenes* has been reported in various studies using liquid food products such as milk (skim or whole), juices, and liquid whole egg (Komora et al., 2020; Pathanibul et al., 2009; Pokhrel et al., 2019; Ponce et al., 1998). For example, Black et al. (2005) observed a >3 log reduction in *L. innocua* as synergistic effect when skim milk was treated with HHP (500 MPa, 5 min) combined with nisin (500 IU/mL). Additionally, Oner (2020) reported that in avocado dressing, HHP (400 MPa, 3 min) combined with nisin at concentrations 10-fold higher than those used in the present study (100 or 200 ppm) increased the reduction of *L. innocua* from 1.8 (HHP alone) to 3.5 log (HHP + nisin) whereas nisin alone achieved only a 0.5–0.6 log reduction. However, this is the first study to investigate this combination in a PBMA for the control of *L. monocytogenes*, and more importantly, the first to investigate the inactivation kinetics of HHP combined with nisin against this pathogen in any food matrix.

In contrast, for the 10403S strain, the HHP + nisin treatment resulted in a statistically significant decrease in the time required to achieve 3-log reduction only at 400 MPa ($p < 0.05$), with the $3D_P$ value dropping from 6.37 ± 0.55 min (HHP alone) to 2.57 ± 0.28 min (HHP + nisin). This indicates that pressure of at least 400 MPa is necessary for the combined treatment to effectively enhance the inactivation of this strain. The observed difference may be attributed to strain-dependent pressure and nisin resistance. Notably, LO28 showed significantly lower $3D_P$ values than 10403S at both 350 MPa (7.65 ± 0.42 vs. 10.91 ± 0.83min) and 400 MPa (2.16 ± 0.07 vs. 6.37 ± 0.55 min) under HHP alone ($p < 0.05$). Strain-related variability has also been reported in previous studies combining HHP with chitosan in ACES buffer (Giannoulis & Karatzas, 2024).

In this respect, as a representative example of the synergistic effect between HHP and nisin, the inactivation data (log N/N_0) at 400 MPa for 3 min further confirmed the previously observed results from the estimated $3D_P$ values. Specifically, *L. monocytogenes* LO28 cell counts were reduced by 4.73 ± 0.09 log under HHP + nisin, compared to a 3.71 ± 0.27 log reduction with HHP alone. In contrast, a lower overall reduction was observed for strain 10403S under the same conditions due to its higher resistance to HHP; however, the difference between treatments was more evident (2.27 ± 0.21 log for HHP alone vs. 3.8 ± 0.2 log for HHP + nisin). Although these reductions demonstrate a clear synergistic effect, HHP treatment in the range applied in the present study generally do not achieve comparable reductions (≥8–10 log) obtained during milk pasteurization under legal time-temperature conditions (e.g. 72 °C for 15 s) which were used as the reference for pasteurization equivalence in the EFSA opinion (EFSA Panel on Biological Hazards et al., 2022). Nevertheless, HHP is expected to better preserve, or even improve at suitable pressure levels, the viscosity and flavor characteristics while limiting β-glucan degradation compared with conventional heat treatment of oat beverages, as reported previously (Ahmad et al., 2025; McCarron et al., 2025).

3.2. Impact of pressure increase in L. monocytogenes inactivation kinetics by HPP with and without nisin

The impact of pressure intensity on the primary inactivation kinetics is shown in Fig. 4. A clear linear relationship (Bigelow-type model) was observed between the log-transformed $3D_P$ values (log $3D_P$) and applied pressure levels for both *L. monocytogenes* strains (LO28 and 10403S), under both treatment conditions (HHP alone and HHP + nisin). Therefore, the Bigelow-type model (Eq. (7)), was used to fit the data and quantitatively characterize the effect of pressure through the z_P parameter. The negative linear correlation confirms that increasing the pressure leads to faster inactivation of both *L. monocytogenes* strains, as indicated by the decreasing log $3D_P$ values.

The z_P value reflects a key aspect of bacterial piezotolerance, representing the pressure increase required to reduce the log $3D_P$ by a factor of 10. Using the secondary model (Eq. (7)), z_P and log $3D_{Pref}$ values were calculated for each *L. monocytogenes* strain, treatment, and replicate (Table 3). Statistical analysis revealed significant differences ($p < 0.05$) between the two strains under HHP treatment alone. Specifically, the z_P for strain LO28 was 102.7 ± 2.7 MPa, whereas for 10403S it was substantially higher at 193.8 ± 10.6 MPa, indicating a greater piezotolerance. These findings are consistent with previous studies, despite differences in food matrices and modeling approaches. Reported z_P values for *L. monocytogenes* by Chen and Hoover (2004); Hereu et al. (2012); Lerasle et al. (2014) range from 105 to 163 MPa, aligning with the values observed in this study. The strain-dependent response of different foodborne pathogens to HHP (e.g. *L. monocytogenes, Salmonella* spp and *E. coli*) has also been documented by Guillou and Membré (2019), and Torrents-Masoliver, Jofré, et al. (2025) further highlighting the variability in pressure tolerance among strains.

Furthermore, z_P values confirmed that the effect of nisin was considered as a significant factor influencing *L. monocytogenes* inactivation during HHP treatment particularly in the pressure-tolerant 10403S strain where a pronounced and statistically significant ($p < 0.05$) decrease in z_P was observed (from 193.8 to 107.4 MPa). Although the reduction in $3D_P$ values with nisin was more evident in LO28, the statistically significant ($p < 0.05$) decrease in z_P observed for 10403S indicates that this strain, initially more resistant to HHP alone, was more affected (compared to LO28) to pressure increase in the presence of nisin. More specifically, the pressure required to reduce the log $3D_P$ by one log in 10403S decreased by nearly 100 MPa in the presence of nisin. Even if nisin alone did not have any significant effect in either strain, one possible explanation is that 10403S may be more susceptible to nisin than LO28, potentially due to differences in membrane composition or stress response pathways. As a result, the combination of nisin and HHP exerts a stronger effect on the piezotolerance of strain 10403S than on strain LO28. These findings highlight that the efficacy of HHP can be influenced by the presence of nisin, and that its impact depends on the specific *L. monocytogenes* strain.

It is also worth noting that the secondary model provided an excellent fit to the experimental data, as evidenced by the high adjusted R^2_{adj} values (>0.94) and low RMSE and RSS values across all treatments and strains (Table 3), supporting the suitability of the Bigelow-type model to describe the pressure dependence of log $3D_P$ values.

3.3. Industrial relevance

From an industrial perspective, the careful selection of bacterial strains is essential for validating HHP treatment under realistic worst-case conditions, thereby ensuring the safety of food products. As previously discussed, strain-specific variability had a substantial impact on the efficacy of HHP, both alone and in combination with nisin. The two *L. monocytogenes* strains evaluated in this study (LO28 and 10403S) exhibited different inactivation behaviors in the oat-based PBMA, with 10403S showing greater resistance to treatment. Therefore, for industrial validation purposes in oat-based milk alternatives subjected to
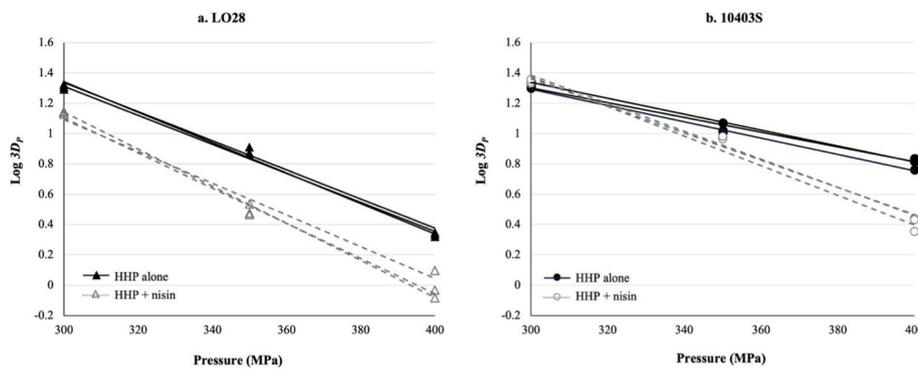
Fig. 4. Relationship between pressure and log $3D_P$ values of *L. monocytogenes* **(a)** LO28 and **(b)** 10403S in oat PBMA, with and without the addition of nisin (500 IU/mL), during HHP treatment. Symbols represent the mean log $3D_P$ values for HHP alone (▲ - LO28; ● - 10403S) and HHP + nisin (△ - LO28; ○ - 10403S). Lines represent the linear regression fits (Bigelow-type model).

Table 3
Parameter estimates and goodness of fit of the secondary modelling (Bigelow type; Eq. (7)) to each replicate data set (consisting of three primary kinetic curves) for HHP inactivation of *L. monocytogenes* LO28 and 10403S strains under HHP alone or combined with nisin. Mean and standard deviation of the estimated parameters are also provided in bold.

L. monocytogenes strain	Treatment	Replicate	$\log 3D_{ref}$ (log min)		z_P (MPa)		RSS	RMSE	R^2_{adj}
LO28	HHP	1	0.34	**0.36 ± 0.02**	99.6	ᵇᶜ**102.7 ± 2.7**	0.0016	0.0403	0.9936
		2	0.38		104.1		0.0045	0.0668	0.9808
		3	0.35		104.4		0.0018	0.0428	0.9921
	HHP + nisin	1	0.04	**−0.03 ± 0.07**	95.5	ᶜ**87.8 ± 6.9**	0.0161	0.1268	0.9430
		2	−0.06		86.3		0.0034	0.0585	0.9899
		3	−0.08		81.8		0.0000	0.0057	0.9999
10403S	HHP	1	0.75	**0.79 ± 0.03**	185.4	ᵃ**193.8 ± 10.6**	0.0003	0.0166	0.9962
		2	0.82		205.8		0.0022	0.0466	0.9639
		3	0.81		190.3		0.0000	0.0059	0.9995
	HHP + nisin	1	0.40	**0.44 ± 0.04**	102.5	ᵇ**107.4 ± 4.6**	0.0123	0.1111	0.9494
		2	0.47		111.5		0.0038	0.0613	0.9815
		3	0.46		108.2		0.0051	0.0716	0.9762

Superscript letters (a-c) indicate statistically significant differences for z_P mean within the two *L. monocytogenes* strains (LO28, 10403S) and two HHP treatments (HHP alone, HHP + nisin). Values that do not share a letter are significantly different (Tukey's HSD test; $p < 0.05$).
RMSE: Root mean sum of squared error; RSS: Residual Sum of Squares; R^2_{adj}: adjusted coefficient of determination.

HHP, the use of 10403S is recommended as the worst-case scenario.

Finally, it is well-known that HHP presents several economic challenges that impact its broader adoption in the food industry. While this technology offers considerable benefits in terms of food safety and quality preservation, its implementation is often constrained by substantial capital investment requirements for equipment and elevated operational costs (Cacace et al., 2020). In the present study, the observed decrease in z_P values under combined HHP and nisin treatment suggests that smaller pressure increments are sufficient to significantly increase microbial inactivation compared to HHP alone. This has important industrial implications: optimizing processing conditions through synergistic combinations (e.g., with nisin) allows manufacturers to achieve microbial safety targets at lower pressures or shorter treatment times, thus reducing energy consumption, equipment wear, and processing time. Such optimizations not only contribute to cost-efficiency but also help preserve the sensory and nutritional properties of plant-based milk alternatives, supporting product quality and consumer acceptance.

It should nevertheless be noted that the current study focused primarily on microbial inactivation behavior and its modelling under HHP and nisin. Therefore, further characterization of physicochemical properties (such as viscosity, stability, and color) as well as key nutritional attributes would be valuable to better understand the impact of the HHP-nisin combination on overall product quality. In addition,

sensory evaluation and consumer acceptance studies would help confirm that processing conditions effective against *L. monocytogenes* remain aligned with product quality expectations and market feasibility.

4. Conclusions

This study demonstrated for first time a quantitative characterization of the effect of HHP alone or combined with nisin on the inactivation of *L. monocytogenes* in an oat-based milk alternative. The application of primary kinetic modeling for $3D_P$ estimations offers critical insights for risk assessment and process optimization. The observed strain-specific differences in pressure sensitivity, both with HHP alone and in combination with nisin, underscore the importance of selecting appropriate challenge strains, with 10403S identified as a suitable worst-case strain for validation studies. Nisin significantly enhanced microbial inactivation during HHP, particularly by reducing the pressure resistance (z_P) of the more tolerant strain, 10403S. These findings support the development of optimized HHP treatment conditions and contribute to the safe production of refrigerated, clean-label, minimally processed oat-based milk alternatives. In addition, the greater reduction in surviving cells achieved with nisin may delay spoilage onset, potentially extend the shelf life and reduce food waste compared to standard formulations. Nonetheless, the potential recovery of sublethally injured cells during refrigerated storage remains a concern which needs to be further

explored. Future studies should also assess sensory attributes and consumer acceptance to ensure that HHP-treated oat PBMA products meet consumer expectations.

CRediT authorship contribution statement

Nikolaos Giannoulis: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Berta Torrents-Masoliver:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Sara Bover-Cid:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Vasilis P. Valdramidis:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Kimon Andreas G. Karatzas:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119038.

Data availability

Data will be made available on request.

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