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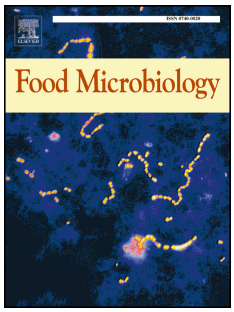

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Growth and growth boundary model with terms for melting salts to predict growth responses of *Listeria monocytogenes* in spreadable processed cheese

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ABSTRACT

The aim of this study was to develop and validate a growth and growth boundary model with terms for melting salts to predict growth of *Listeria monocytogenes* in spreadable processed cheese. Cardinal parameter terms for phosphate salts and citric acid were developed in broth studies and used to expand an available growth and growth boundary model. The expanded model includes the effect of nine environmental factors (temperature, pH, a_w, lactic acid, acetic acid, citric acid, orthophosphate, di-phosphate and tri-phosphate). To generate growth data for model evaluation challenge tests with inoculated commercial (n = 10) and customized (n = 10) spreadable processed cheeses were performed. Evaluation of the new model by comparison of observed and predicted μ_{max}-values resulted in a bias factor of 1.12 and an accuracy factor of 1.33 (n = 42). Prediction of growth and no-growth responses in processed cheese (n = 60) were 89% correct with 11 % fail-safe and 0 % fail-dangerous predictions. The developed model can be used to support product development, reformulation or risk assessment for spreadable processed cheese.

Keywords: Phosphate salts, product development, risk assessment, predictive microbiology

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1. Introduction

Spreadable processed cheese is a ready-to-eat product manufactured by blending cheese, melting salts (e.g. sodium or potassium salts of phosphoric or citric acid) and other dairy and non-dairy ingredients, followed by heating and mixing to obtain a uniform molten mass which is typically hot-filled into the final packaging (Fox et al., 2017). Formulation parameters for spreadable processed cheese may vary considerably in terms of pH (4.7-6.3), moisture (ca. 50-70%) and salt content (Maurer, 2012; Kim et al., 2018). Food-grade hydrocolloids (e.g. carob bean gum, guar gum, xanthan gum, gelatine and/or carrageenan) can be used to influence product texture and to reduce the water activity of spreadable processed cheese (Guinee et al., 2004). Melting salts are ingredients known to contribute to the microbiological safety and stability of spreadable processed cheese, besides their main function as emulsifying agents. Among melting salts, phosphates are well known to inhibit the growth of spore-forming bacteria which are key microorganisms to control in processed cheeses (Tanaka et al., 1986; Tompkin, 1983). However, little information is available about their inhibitory effect against pathogens that may potentially contaminate the product during open shelf-life and especially under conditions of temperature abuse by the consumer.

Unsafe food handling by consumers, including cross-contamination and storage conditions, is believed to contribute significantly to foodborne illness (De Jong et al., 2008; Evans and Redmond, 2018; Redmond and Griffith, 2003). Based on data for several countries, more than one third of domestic refrigerators operate at temperatures above 5°C which is the maximum recommended chilled temperature for most ready-to-eat products (James et al., 2008; Roccato et al., 2017; WHO, 2006). Hygiene and temperature control can be critical in relation to food safety with EFSA estimates showing that prevention of growth of *Listeria monocytogenes* in ready-to-eat products at the consumer phase can reduce annual listeriosis cases in the Member States by 37% (EFSA, 2018).

Within the EU, it is mandatory for food business operators to evaluate and manage potential *L. monocytogenes* growth depending on product characteristics and different reasonably foreseeable storage conditions of ready-to-eat foods (EC, 2005). Melting salts are known to inhibit growth of foodborne pathogens such as *Bacillus cereus*, *Clostridium botulinum* and *Staphylococcus aureus* (ter Steeg et al., 1995a; Maier et al., 1999; Loessner et al., 1997). In the same way, melting salts may be important in controlling *L. monocytogenes* growth in spreadable processed cheeses but their anti-listerial effect remains little studied. The potential growth of *L. monocytogenes* e.g. after opening a hot-filled packaged food product can be evaluated by challenge tests or predictive mathematical modelling (EC, 2005). Application of validated predictive models is typically a faster and more cost effective approach but to accurately predict growth responses of *L. monocytogenes* mathematical models must include the effect of all important preserving factors (Mejlholm et al., 2010; Ross and Dalgaard, 2004). Many *L. monocytogenes* growth models are available, some including the inhibitory effect of several intrinsic along with extrinsic factors and a few models have been successfully validated for different types of dairy products (Augustin et al., 2005; Martinez-Rios et al. 2019; Mejlholm et al., 2010). However, none of the available *L. monocytogenes* growth models include the inhibitory effect of melting salts or have been successfully validated for spreadable processed cheeses.

The objective of the present study was to expand and validate a mathematical model to predict growth and growth boundary of *L. monocytogenes* in spreadable processed cheese including phosphate/citrate salts and/or organic acids. Firstly, the growth inhibiting effects of phosphate and citrate salts on *L. monocytogenes* were studied in broth and their minimum inhibitory concentrations (MICs) were determined. Secondly, new mathematical terms for the inhibiting effect of these compounds were included in the growth and growth boundary model of Mejlholm and Dalgaard

(2009). Finally, the performance of the expanded model was evaluated by comparison of predicted and observed growth for *L. monocytogenes* in spreadable processed cheese.

2. Materials and methods

2.1. Bacterial strains and pre-culture conditions

Four dairy related strains of *L. monocytogenes* were provided by Arla Foods amba and used as a cocktail (SLU 92, 612, LM19, 6) to determine μ_{max}-values in broth and for inoculation of challenge test. Prior to use, each strain was transferred from storage at -80°C to Brain Heart Infusion (BHI) broth (CM1135, Oxoid, Hampshire, UK) and incubated for 24h at 25°C. Subsequently, for broth studies the individual strains were pre-cultured one day at 25°C in BHI broth with pH 6.2 and 0.5% NaCl. For challenge testing the individual stains were pre-cultured one or two days at 8°C to 20°C in BHI broth with pH 6.2 and 1% NaCl to simulate temperature abuse conditions encountered in spreadable processed cheese during open-shelf life. Pre-cultures were grown to a relative increase in absorbance (540 nm) of 0.05 to 0.2 (Novaspec II, Pharmacia Biotech, Allerød, Denmark). The *L. monocytogenes* cocktail of strains (*Lm*-mix) used in broth and challenge test studies were produced by mixing equal volumes of individual pre-cultures. The cell concentration of *Lm*-mix was determined by direct phase-contrast microscopy at 1000x magnification considering that one cell per field of view corresponded to a concentration around 10^6 cfu/ml (Adams and Moss, 2016).

2.2. Phosphate and citrate salts

105 MICs were determined for three different phosphate salts and trisodium citrate.
106 Furthermore, the anti-listerial effect of eight commercially available melting salts preparations were
107 determined (Table 1).

108

109 2.3. Cardinal parameter values for phosphate and citrate salts

110 The inhibitory effect of eight to 17 different concentrations of P1 (0 to 6.5%), P2 (0 to 6%),
111 P3 (0 to 5%) and TC (0 to 9%; corresponding to 0-137,000 ppm of citric acid) on *Lm*-mix were
112 determined at 25°C in BHI-broth with pH 6.2. A total of 154 μ_{max}-values were determined. For each
113 condition, growth of *Lm*-mix was determined in duplicate by using automated absorbance
114 measurements at 540 nm (BioScreen C, Labsystems, Helsinki, Finland). Detection times, defined as
115 incubation time necessary to observe an increase in absorbance of 0.05 from the lowest absorbance
116 measured in the beginning of incubation, were determined for each absorbance growth curve. μ_{max}-
117 values of *Lm*-mix were determined from absorbance detection times for serially diluted inoculation
118 levels of 10^2, 10^3, 10^4, 10^5 and 10^6 cfu/ml as previously described (Dalgaard and Koutsoumanis,
119 2001). The cardinal parameter values for the different phosphate and citrate salts (P1, P2, P3 and
120 TC) were estimated by fitting eq. (1) to square root transformed μ_{max}-values of *L. monocytogenes*.

121 $$\sqrt{\mu_{max}} = \sqrt{\mu_{ref}{}_{25°C} \cdot \left(1 - \left(\frac{[P \text{ or } TC]}{MIC_{P \text{ or } TC}}\right)^{n1}\right)^{n2}} \quad (1)$$

122 where [P or TC] are the concentrations (%) of individual phosphates (P1, P2, P3) or citrate salt (TC)
123 and $MIC_{P \text{ or } TC}$ is the fitted MIC-value (%) of individual phosphates (P1, P2, P3) or citrate salt (TC)
124 that prevents growth of *L. monocytogenes*. The cardinal parameter value for citric acid ($MIC_{U\ CAC}$)
125 was determined from concentrations of undissociated citric acid calculated by eq. (2) with a pKa

value of 3.13 (Ross and Dalgaard, 2004) from concentrations of TC. The cardinal parameter value was estimated by fitting eq. (3) to square root transformed μ_{max}-values of *Lm*-mix.

$$Undissociated\ organic\ acid\ (mM) = \frac{Citric\ acid\ (mM)}{1+10^{pH-pK_a}} \quad (2)$$

$$\sqrt{\mu_{max}} = \sqrt{\mu_{ref_{25°C}} \cdot \left(1 - \left(\frac{[\,CAC_U\,]}{MIC_{U\ CAC}}\right)^{n1}\right)^{n2}} \quad (3)$$

where [CAC_U] is the concentration (mM) of undissociated citric acid and $MIC_{U\ CAC}$ is the fitted MIC-value of undissociated citric acid that prevents growth of *L. monocytogenes.* When fitting eq. (1 and 3), n1 was set to 0.5 or 1 and n2 was set to 1 or 2 (Dalgaard, 2009) in order to describe data most appropriately and this was determined from root mean square error (RMSE) values.

2.3.1. Growth inhibiting effect of interaction between phosphate and citrate salts

The effect of interaction for different combinations of phosphate and citrate salts concentrations (P1: 0-6%; P2: 0-5.5%; P3: 0-5%; TC: 0-8%) on *Lm*-mix were determined in BHI-broth with pH 6.2 at 25°C. A total of 66 μ_{max}-values were generated experimentally as explained above (Section 2.3). Experiments were designed to include combinations of concentrations that were close to the growth boundary of *L. monocytogenes*.

2.3.2. Anti-listerial effect of commercial melting salt preparations

The inhibitory effect for different concentrations of DP (0 to 8.6%), BUDAL (0 to 15.5%), PZ6, PZ35, S9, PZ189 (0 to 4.5%) and S9K (0 to 8%) on μ_{max}-values of *Lm*-mix were determined. A total of 94 μ_{max}-values were generated in BHI-broth with pH 6.2 at 25°C (See 2.3).

145

146 2.4. Development of a new *L. monocytogenes* growth and growth boundary model

147 New mathematical terms including MIC values for P1, P2, P3 and either TC or CAC_U were
148 added to an existing cardinal parameter growth and growth boundary model previously validated
149 for growth of *L. monocytogenes* in some non-fermented dairy products (Mejlholm and Dalgaard,
150 2009; Mejlholm et al., 2010). Of the 12 environmental factors in that model, exclusively terms for
151 the effect of temperature, pH, NaCl/a_w, lactic acid and acetic acid were used in the present study
152 and included in a new *L. monocytogenes* growth and growth boundary model with terms for the
153 inhibitory effect of phosphate salts and either citrate salt or undissociated citric acid (eq. 4). A
154 recently developed cardinal parameter pH_{min}-function was used to estimate pH_{min}-values for *L.*
155 *monocytogenes* depending on the storage temperature (Martinez-Rios et al., 2019).

156 $$\mu_{max} = \mu_{ref} \cdot \left[\frac{(T+2.83)}{(T_{ref}+2.83)}\right]^2 \cdot \frac{(a_w-0.923)}{(1-0.923)} \cdot \left[1-10^{(pH_{min}-pH}\right] \cdot \left(1-\frac{[LAC_U]}{3.79}\right) \cdot \left(1-\sqrt{\frac{[AAC_U]}{10.3}}\right) \cdot$$

157 $$\left[\left(1-\left(\frac{[CAC_U]}{MIC_{U\,CAC}}\right)\right) or \left(1-\left(\frac{[TC]}{[MIC_{TC}]}\right)^{n1}\right)^{n2}\right] \cdot \left(1-\left(\frac{[P1]}{[MIC_{P1}]}\right)^{n1}\right)^{n2} \cdot \left(1-\left(\frac{[P2]}{[MIC_{P2}]}\right)^{n1}\right)^{n2} \cdot$$

158 $$\left(1-\left(\frac{[P3]}{[MIC_{P3}]}\right)^{n1}\right)^{n2} \cdot \xi \qquad (4)$$

159 where μ_{ref} is a fitted parameter with value equal to μ_{max} at the reference temperature (T_{ref}) of 25°C
160 when other environmental factors do not inhibit growth; T is the temperature (°C) and a_w is the
161 water activity measured in the product (Supplementary Table S1). [LAC_U], [AAC_U], [CAC_U] are
162 the concentrations (mM) of undissociated lactic acid, acetic acid and citric acid in the water phase,
163 respectively. [P1], [P2], [P3] and [TC] are the concentrations (%) in water phase of orthophosphate,
164 di-phosphate, tri-phosphate and trisodium citrate respectively. [MIC_{P1}], [MIC_{P2}], [MIC_{P3}], and
165 [MIC_{TC}] are the fitted MIC-values (%, in the water phase) of orthophosphate, di-phosphate, tri-

166 phosphate and trisodium citrate, respectively, that prevents growth of *L. monocytogenes*. The
167 interaction between environmental parameters (ξ) was modelled as previously described using the
168 Le Marc approach (Le Marc et al., 2002; Mejlholm and Dalgaard, 2009). The effect of interaction
169 between environmental factors in eq. (4) was expressed by the parameter ξ, which has a value of
170 between 0 and 1. The value of ξ, was calculated according to eq. (5), with contributions from
171 different environmental factors as shown in eq. (6) and (7). In eq. (7), e_i represents the
172 environmental factors. Eq. (5) divides the space of environmental factors into three regions: (i) if ψ
173 is less than 0.5, then no interactive effect between environmental factors occurs (ξ =1); (ii) if ψ is
174 greater than 1, then no growth occurs (ξ =0); and (iii) if ψ is less than 1 and greater than 0.5, then
175 the growth rate (μ_{max}, 1/h) is reduced depending on the value of ψ. A ψ value greater than 1 (e.g.,
176 1.5 or 2.0) provides a measure of how far the properties of a specific food product is from the
177 predicted growth boundary of *L. monocytogenes* (Mejlholm and Dalgaard, 2009).

178 $\xi(\varphi(\mathrm{T}, a_w, \mathrm{pH}, [\mathrm{LAC}_U], [\mathrm{AAC}_U], [\mathrm{CAC}_U], [\mathrm{P1}], [\mathrm{P2}], [\mathrm{P3}], [\mathrm{TC}]))$

179 $$= \begin{cases} 1, & \psi \leq 0.5 \\ 2(1-\psi), & 0.5 < \psi < 1 \\ 0, & \psi \geq 1 \end{cases} \quad (5)$$

180 where $\xi(\varphi(\mathrm{T}, a_w, \mathrm{pH}, [\mathrm{LAC}_U], [\mathrm{AAC}_U], [\mathrm{CAC}_U], [\mathrm{P1}], [\mathrm{P2}], [\mathrm{P3}], [\mathrm{TC}]))$ is the term describing the
181 effects of interactions between environmental factors on μ_{max}:

182 $$\varphi T = \left[1 - \sqrt{(T + 2.83)/(T_{ref} + 2.83)}\right]^2$$

183

$$\varphi a_w = \left[1 - \sqrt{(a_w - 0.923)/(1 - 0.923)}\right]^2$$

$$\varphi pH = \left[1 - \sqrt{1 - 10^{(pH_{min} - pH)}}\right]^2$$

φ[LAC]; [AAC]; [CAC]

$$= \left[1 - \left(\left(1 - \sqrt{[LAC_U]/3.79}\right) \cdot \left(1 - \sqrt{[AAC_U]/10.3}\right) \cdot (1 - [CAC_U]/MIC_{U\,CAC})\right)\right]^2$$

φ[P1]; φ [P2]; φ [P3]; φ [TC]

$$= \left[1 - \left(\left(1 - \left(\frac{P\ or\ TC}{MIC_{P\ or\ TC}}\right)^{n1}\right)^{n2}\right)\right]^2 \quad (6)$$

$$\psi = \sum_i \frac{\varphi_{e_i}}{2\prod_{j \neq i}\left(1 - \varphi_{e_i}\right)} \quad (7)$$

The inhibiting effect of organic acids on interaction with other environmental factors in eq. (6) was modelled by multiplication of their effects as previously suggested (Coroller et al., 2005).

2.5. Challenge test with spreadable processed cheese

To generate data for model evaluation, growth of *L. monocytogenes* in spreadable processed cheese was determined in 20 inoculated challenge tests including 60 growth/no-growth responses at constant and dynamic storage temperatures (see section 2.6.). These included ten batches/formulations of customized spreadable processed cheese and 4 batches of commercially available spreadable processed cheese (Table 2).

2.5.1. Product characteristics

Ten customized spreadable processed cheese recipes were designed to evaluate the effect of phosphate salts, citrate salt and undissociated citric acid. The customized recipes were produced in the pilot plant at Arla Innovation Centre in Aarhus and transported on ice to DTU Food where they were stored upon arrival at 2°C for a maximum of 48h until further studied. Individual batches of customized spreadable processed cheese were produced with 3% or 6% orthophosphate (P1), di-phosphate (P2) or trisodium citrate (TC) and 2% or 5% tri-phosphate (P3). A commercially available emulsifying salt preparation (S9K) was used to produce spreadable processed cheese with two different concentrations (3% or 6%). Two commercial spreadable processed cheeses were obtained from a local supermarket. Product pH was measured with a PHM 250 Ion Analyzer (MetroLab^{TM}, Radiometer, Copenhagen, Denmark) after 1h stirring of a 5 g sample in 25 ml of distilled water. NaCl was quantified by automated potentiometric titration (785 DMP Titrino, Metrohm, Hesisau, Switzerland) and a_w was calculated from the concentration of NaCl in the water phase (%WPS) according to the relation derived from Resnik and Chirife (1988) ($a_w = 1-0.0052471 \cdot \%WPS - 0.0002206 \cdot \%WPS^2$). In addition a_w was measured by a water activity meter (Aqua Lab model CX-2, Decagon devices Inc., Pullman, US) (Supplementary Table 1). The concentrations of lactic, acetic and citric acid were determined by HPLC using external standards for identification and quantification. In order to improve extraction, a centrifugation step was applied (Dalgaard and Jørgensen, 2000; Martinez-Rios et al., 2016). Phosphate and citrate salt concentrations were determined by Eurofins, New Orleans, USA (test method QA02S). Product characteristics were determined on three samples for each batch and data reported as average ± standard deviation.

2.5.2. Inoculation, storage conditions and microbiological analysis

223 Cheese was inoculated with 0.1% (v/w) of *Lm*-mix appropriately diluted in chilled saline
224 water (0.85% NaCl) to obtain an initial concentration in the range of 1-3 Log cfu/g. Following
225 inoculation, 50±5g of cheese was placed in containers similar to those used for commercial
226 distribution of the product. Samples were stored at 5, 10, 15 and 22°C or under dynamic
227 temperatures (Table 2). Storage temperature was recorded every 30 minutes by data loggers
228 (TinytagPlus, Gemini Data Loggers Ldt., Chichester, UK). Storage time was from 8 to 83 days for
229 different treatments with 7 to 27 sampling times per treatment.

230 At each time of sampling a container with 50±5g of cheese was analysed and then
231 discarded. 10 g of cheese was diluted 10-fold in chilled physiological saline water with peptone
232 (0.85% NaCl, 0.1% Bacto™ Peptone, 211677, BD Bioscience, San Jose, USA) and subsequently
233 homogenized for 30s at normal speed in a stomacher (Stomacher 400 Circulator, Seward Medical,
234 London, UK). Viable counts of *L. monocytogenes* were determined by surface plating on Palcam
235 agar base (CM0877, Oxoid, Basingstoke, UK) with selective supplement (SR0150, Oxoid) and
236 incubated at 37°C for 48h.

237

238 2.5.3. Primary growth model

239 The integrated and log transformed logistic model with delay (four parameter model) or
240 without delay (three parameter model) (eq. (8); Rosso et al., 1996) was fitted to all individual
241 growth curves of *L. monocytogenes* obtained in challenge tests at constant temperatures. Fitted
242 parameter values for initial cell concentration (Log N_0, Log cfu/g), lag time (t_{lag}, h), maximum
243 specific growth rate (μ_{max}, 1/h) and maximum population density (Log N_{max}, Log cfu/g) were
244 determined for each growth curve and data was reported as average ± standard deviation for
245 challenge tests (Table 2). An F-test was used to determine if the lag time was significant.

$$\text{Log } (N_t) = \text{Log } (N_0) \qquad if\ t < t_{lag}$$

$$\text{Log } (N_t) = Log\left(\frac{N_{max}}{1+\left(\left(\frac{N_{max}}{N_0}\right)-1\right)\cdot\exp\left(-\mu_{max}\cdot(t-t_{lag})\right)}\right) \qquad if\ t \geq t_{lag} \quad (8)$$

where t is the storage time (h) and N_t is the cell concentration (cfu/g) at time t. Other parameters were indicated above.

The relative lag time ($RLT = t_{lag} \cdot \mu_{max}/\ln(2)$) (Mellefont and Ross, 2003) was calculated for all growth curves of *L. monocytogenes* in challenge tests (Table 2). It was evaluated if RLT-values were constant ($RLT = K_1$) or dependent on storage temperature ($RLT = K_1 + K_2/T^2$) as reported by Hereu et al. (2014).

2.6. Evaluation of the new *L. monocytogenes* growth and growth boundary model

Comparison of observed and predicted μ_{max}-values was carried out by calculation of bias (B_f) and accuracy (A_f) factor values (Ross, 1996). For pathogenic bacteria, $0.95 < B_f < 1.11$ indicates a good model performance, with B_f 1.11-1.43 or 0.87-0.95 corresponding to acceptable model performance and B_f <0.87 or >1.43 reflecting unacceptable model performance (Mejlholm et al., 2010). A_f > 1.5 has been suggested to indicate an incomplete model or systematic deviation between observed and predicted μ_{max}-values (Mejlholm and Dalgaard, 2013). Firstly, we used this approached to evaluate the effect of interaction among environmental factors (eq. (4)). Secondly, the approach was applied to evaluate if the new model could appropriately predict the inhibitory effect of commercial melting salt preparations on the growth of *L. monocytogenes*. For these predictions the concentrations of individual phosphates in the commercial melting salt preparations were analysed and concentrations of P1, P2 and P3 were used as model input to obtain predictions

(Table 5). Finally, the performance of the new model was evaluated by comparing predicted and observed growth responses in 20 challenge tests with spreadable processed cheese (see section 2.5). Predicted and observed growth and no-growth responses were assessed by calculating the percentage of all samples that were correctly predicted with or without inclusion in eq. 4 of the term for interaction between environmental factors (ξ). Incorrect predictions were considered as fail-safe (growth predicted with no growth observed) or fail-dangerous (no growth predicted with growth observed). ψ–values (eq. (5)) was used to describe how far the predicted response (growth or no-growth) was from the growth boundary (ψ =1).

The acceptable simulation zone (ASZ) approach was used to compare observed and predicted growth in challenge tests were growth was observed under constant or dynamic temperature storage. The acceptable interval was defined as +0.5 and -1.0 Log cfu/g from the simulated growth of *L. monocytogenes*. When at least 70% of the observed values were within ASZ, the simulation was considered acceptable (Oscar, 2007; Velugoti et al., 2011).

2.7. Evaluation of existing models

Three existing *L. monocytogenes* growth models were evaluated to assess their ability to predict growth responses in spreadable processed cheese. The studied models were: (a) the model of Mejlholm and Dalgaard (2009) previously evaluated for different non-fermented dairy products (Mejlholm et al., 2010), (b) the model of Augustin et al. (2005) developed for cheese and including terms for temperature, pH, NaCl/a_w, phenol, nitrite and CO_2 and (c) the ComBase model including the effect of temperature, pH, NaCl/a_w and lactic acid (Combase, 2012).

2.8. Statistical analysis and curve fitting

288 Model parameters and standard errors were estimated by using GraphPad PRISM (version 8,
289 GraphPad Software, San Diego, CA, USA). F-tests to determine significant lag times were
290 performed using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA).

291

292 **3. Results**

293

294 3.1. Development of a new *L. monocytogenes* growth and growth boundary model

295 The fitted MIC-values for phosphates were 14.9±1.1 %, 9.4±0.4 %, 7.6±0.2 % in the water
296 phase for orthophosphate (P1), di-phosphate (P2), tri-phosphate (P3), respectively (Fig. 1; Table 4).
297 The fitted MIC-values for trisodium citrate (TC) salts and undissociated citric acid (CAC_U) were
298 11.0±0.3 % and 0.75±0.02 mM, respectively (Fig. 2; Table 3). These MIC-values were used in eq.
299 (4) together with a μ_{ref}-value of 0.419 1/h as determined at 25°C by Mejlholm and Dalgaard (2009).
300 When predictions by the new model (Eq. (4)) were performed either the MIC-value for TC or
301 CAC_U was used.

302 3.2. Challenge test with spreadable processed cheese

303 Commercial spreadable processed cheese showed little variation in initial pH (6.1-6.3), a_w
304 (0.969-0.975) or concentrations of P1 (1.90-2.14%) and TC (0.49-0.58%) (Table 2). More
305 variability was observed for water phase concentrations of lactic acid (4120-12,624 ppm), acetic
306 acid (619-1,594 ppm) and citric acid (518-7,708 ppm) (Table 2).

307 *L. monocytogenes* grew in commercial spreadable processed cheese at 5, 10, 15 and 22°C
308 and μ_{max}-values were influenced by storage conditions and product characteristics (Table 2 and 3).

As expected storage temperature had a pronounced effect on *L. monocytogenes* growth rate as seen for challenge tests 15, 16 and 17 which were performed with batch 3 of a commercial spreadable processed cheese and therefore had the same product characteristics (Table 2; Table 3). Tri-phosphate (P3) concentration had a major effect on *L. monocytogenes* μ_{max}-values, as suggested by their fitted MIC-values and confirmed by challenge tests 5 and 6 (Table 2, 3 and 4).

3.3. Evaluation of the new *L. monocytogenes* growth and growth boundary model

Broth studies with combinations of phosphates and citrate salt or undissociated citric acid suggested the need to include the growth inhibiting effect of interaction between these factors in the new growth and growth boundary model. By including φ[P1]; φ [P2]; φ [P3]; and either φ [TC] or φ [CAC_U] in eq. (7) the B_f and A_f values changed from 1.55/1.67 to 1.00/1.61. The seven studied commercial melting salt preparations all reduced growth rates of *L. monocytogenes* at 25 °C in BHI broth with pH 6.2 and this growth inhibiting effect was on average acceptably predicted by eq. 4 when using concentrations of P1, P2 and P3 as model input. On average for the 94 μ_{max}-values determined in broth the B_f and A_f were 1.42 and 1.46, respectively (Table 5). Specifically, the model predicted growth with acceptable model performance for DP, PZ35, PZ189 and S9K but overestimates growth for BUDAL and to a lesser extend for PZ6 and S9 (Table 5).

For challenge test with spreadable processed cheese the new model predicted growth rates of *L. monocytogenes* at constant temperature with a good performance as determined from independent growth curves (n= 42) belonging to a total of 14 challenge test where growth was observed (Table 3). Comparison of observed and predicted μ_{max}-values, using either MIC_{TC} (%) or $MIC_{U\ CAC}$ (mM) (Fig. 2; Eq. (1); Eq. (3)), resulted in B_f/A_f –values of 1.06/1.35 or 1.12/1.29, respectively (Table 6). For commercial (n= 27) or customized (n= 15) spreadable processed cheese,

B_f/A_f –values were 1.15/1.33 and 0.91/1.39, respectively, when using MIC_{TC} (%). When using $MIC_{U\,CAC}$ (mM) similar B_f/A_f–values of 1.17/1.36 and 1.05/1.29 were obtained.

RLT-values for growth in spreadable processed cheese showed considerable variability and they were not dependent on storage temperature (Table 3). The minimum, average and maximum *RLT*-values were 0.0, 1.2 and 13.9.

For challenge tests, eq. (4) with interaction between environmental factors (ξ) resulted in 89 % correct prediction of growth and no-growth responses with 11% being fail-safe (Table 3). Without interaction between environmental factors (ξ) 74 % of the growth and no-growth responses were correctly predicted with 26% being fail-safe. Clearly, inclusion of the interaction term (ξ) in eq. (4) was important to accurately predict growth responses of *L. monocytogenes*. The two fail-safe predictions (11%) had ψ-values of 0.3 and 0.4 and these were not close to the growth boundary with (ψ =1). Three correctly predicted no-growth responses had ψ-values of 1.2, 1.5 and 2.4 (Table 3).

On average 58% of the predicted cell concentrations were within the ASZ for spreadable processed cheese when calculated for 15 challenges where growth was observed resulting in 45 growth curves at constant and dynamic temperatures (Table 7, Fig. 3). Predictions were obtained using the minimum observed *RLT*-value for *L. monocytogenes*, N_{max} of 7.9 log cfu/g and the fitted $MIC_{U\,CAC}$–value of 0.75 mM (Table 7, Fig. 3). Lag times had a major effect on the ASZ scores. As examples, challenge test 15 with a significant lag time (306 h, Table 3) resulting in a very low ASZ value (31%), however, when no significant lag time was observed at the same storage temperature (challenge test 12) a ASZ value of 62% was found (Table 7). For challenge test 1 and 15 growth rates were accurately predicted by the model but the presence of lag times resulted in low ASZ scores (Fig.3. a, d). To overcome this limitation of the model, we evaluated the use of average and

maximum RLT-values but results were inferior to those obtained by applying the minimum RLT-values (Table 7).

3.4. Evaluation of existing models

As expected, for spreadable processed cheese with melting salts, unacceptable model performance with B_f–values well above 1.43 were observed for both the model of Mejlholm and Dalgaard (2009) and the ComBase model. Acceptable performance with B_f and A_f of 0.93/1.30 were determined for the model of Augustin et al. (2005) developed for cheese (Table 6).

4. Discussion

A new mathematical model to predict growth and growth boundary of *L. monocytogenes* in spreadable processed cheese was developed by expanding an existing cardinal parameter model with terms to account for the effect of orthophosphate, di-phosphate, tri-phosphate and a new MIC-value for undissociated citric acid of 0.75 mM (Eq.(4)). The new model predicted acceptably the growth at constant and dynamic storage temperatures as well as the growth boundary of *L. monocytogenes* in spreadable processed cheese (Table 6, Table 7, Fig. 3h). The low average ASZ score of 58% was due to significant lag times in some challenge tests and predictions being fail-safe (Fig. 3 a, d). Similar effects of lag times on ASZ scores were previously observed for both *L. monocytogenes* and *Salmonella* spp. (Hereu et al., 2014; Velugoti et al. 2011).

Based on the performed evaluation of the model, its range of applicability included orthophosphate (0.14 to 4.98 %), di-phosphate (<0.01 to 5.09 %), tri-phosphate (<0.01 to 5.17 %), lactic acid (6,371 to 15,328 ppm), acetic acid (568 to 3,483 ppm), citric acid (518 to 38,282 ppm) in the product water phase, pH (6.1 to 6.6), a_w (0.952 to 0.975) and temperature (3.8 to 22.0°C). The

inhibitory effect of several dairy specific ingredients is included in the new model (Eq. (4)) and this makes the model of practical importance for product development, reformulation or risk assessment of spreadable processed cheese. As an example, for a spreadable processed cheese with pH 6.3, a_w 0.972 and water phase organic acid concentrations of 0.8% (lactic acid), 0.1% (acetic acid), 0.3% (citric acid) and 2.0 % (orthophosphate), the predicted time for *L. monocytogenes* to reach the critical concentration of 2 log cfu/g is 4-8 days if this product is contaminated with 1-10 cfu/g by consumer handling, e.g. when opening a package, and then stored at 8°C. A longer open shelf-life or larger safety margin may be desirable and the new model predicts that by substituting the orthophosphate with 2.0 % tri-phosphate the reformulated product requires 13-17 days at 8°C to reach the same critical concentration for *L. monocytogenes*. It seems interesting to apply the new model in combination with available models to predict growth or toxin formation by *C. botulinum* in spreadable process cheese containing melting salts (Glass et al., 2017; Schaffner et al., 1998; ter Steeg and Cuppers, 1995b) to formulate recipes that will inhibit growth of the relevant pathogens. For these applications the new model has the advantages of including the inhibitory effect of ingredients specific to spreadable processed cheese and being validated for these products. When food products are reformulated, product characteristics must be selected at a sufficient distance from the growth boundary so that *L. monocytogenes* does not grow as a consequence of intrinsic variability of product characteristics, storage conditions or strain variability. In this respect, the new model (Eq. (4)) includes the parameter ψ as a quantitative measurement for the distance between specific environmental conditions and the growth boundary of *L. monocytogenes* (ψ =1). As an example, a ψ value of 0.20 was determined by the model for spreadable processed cheese with the following characteristics: pH 6.6, a_w 0.970, 1.0% (lactic acid), 0.1% (acetic acid), 0.2% (citric acid), 0.7% P1, 0.5% P2, 0.6% P3 in the water phase of product and stored at 15°C (Table 2, CT 5). These product characteristics are placed on the growth side of the growth boundary (ψ <1). The new

model can be used to optimize product characteristics to prevent growth of *L. monocytogenes*. The formulation studied in CT 5 can be changed to prevent growth and to obtain a product with a desired ψ-value of e.g. 2. With pH reduced from 6.6 to 5.8, water phase concentrations of lactic acid increased from 1% to 2.2%, acetic acid increased from 0.1% to 0.35% and P1 reduced from 0.7 to 0.3% and P3 increased from 0.6% to 1.5% the predicted ψ-value becomes 2.1.

The model of Augustin et al. (2005) also provided acceptable prediction for growth rates of *L. monocytogenes* in spreadable processed cheese (Table 6) and included the effect of temperature, pH, NaCl/a_w, phenol, nitrite and CO_2. Without terms for organic acids and melting salts the potential of the Augustin et al. (2005) model to contribute to development and reformulation of spreadable processed cheese, however, is limited and in this respect the new model developed in the present study is more performant.

The present study estimated a lower undissociated citric acid MIC-value ($MIC_{U\ CAC}$) for *L. monocytogenes* dairy strains (0.75 mM) than Mejlholm and Dalgaard (2009) observed for seafood isolates (2.21 mM). Future studies should compare model performance when using either $MIC_{U\ CAC}$-values for spreadable processed cheese with lower pH-values than the products evaluated in the present study.

The approach used in the present study to develop an extensive model including the inhibiting effect of both organic acids and phosphate salts could also be interesting for *C. botulinum* as available predictive models with relevance for spreadable processed cheese include few environmental parameters (Glass et al., 2017; Schaffner et al., 1998; ter Steeg and Cuppers, 1995b).

5. Conclusion

423 The present study developed and validated a new model to predict growth and growth
424 boundary of *L. monocytogenes* in spreadable processed cheese. The obtained results demonstrate
425 that interaction among environmental factors improved the performance of the model. This study
426 confirmed that increasing concentrations of phosphate salts reduces the growth of *L. monocytogenes*
427 and therefore, these salts can be used as growth inhibiting compounds. The model can be used to
428 support spreadable processed cheese product development, reformulation or risk assessment. It
429 seems interesting to include the new model in predictive microbiology application software such as
430 the Food Spoilage and Safety Predictor (FSSP http://fssp.food.dtu.dk/) to facilitate prediction of the
431 effect of product characteristics at constant and dynamic temperature storage conditions on growth
432 of *L. monocytogenes* in spreadable processed cheese.
433
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448 **References**

449 Adams, M.R., Moss, M.O., McClure, P.J., 2016. Methods for the Microbiological Examination of
450 Foods. In: Food Microbiology. The Royal Society of Chemistry, Croydon, UK, pp. 413–446.

451 Augustin, J.C., Zuliani, V., Cornu, M., Guillier, L., 2005. Growth rate and growth probability of
452 *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions. Journal
453 of Applied Microbiology 99, 1019–1042. https://doi.org/10.1111/j.1365-2672.2005.02710.x

454 ComBase, 2012. Web-based resource for quantitative and predictive food microbiology.
455 https://www.combase.cc/index.php/en/ Accesed on 6 Febraury 2019

456 Coroller, L., Guerrot, V., Huchet, V., Le Marc, Y., Mafart, P., Sohier, D., Thuault, D., 2005.
457 Modelling the influence of single acid and mixture on bacterial growth. International Journal
458 of Food Microbiology 100, 167–178. https://doi.org/10.1016/j.ijfoodmicro.2004.10.014

459 Dalgaard, P., 2009. Modelling of microbial growth. Bulletin of the International Dairy Federation
460 433, 45–60.

461 Dalgaard, P., Jørgensen, L.V., 2000. Cooked and brined shrimps packed in a modified atmosphere
462 have a shelf life of > 7 months at 0°C, but spoil in 4-6 days at 25°C. International Journal of
463 Food Science and Technology, 35, 431-442. https://doi.org/10.1046/j.1365-2621.2000.00402.x

464 Dalgaard, P., Koutsoumanis, K., 2001. Comparison of maximum specific growth rates and lag
465 times estimated from absorbance and viable count data by different mathematical models.
466 Journal of Microbiological Methods 43, 183–196. https://doi.org/10.1016/S0167-
467 7012(00)00219-0

468 De Jong, A.E.I., Verhoeff-Bakkenes, L., Nauta, M.J., De Jonge, R., 2008. Cross-contamination in
469 the kitchen: Effect of hygiene measures. Journal of Applied Microbiology 105, 615–624.
470 https://doi.org/10.1111/j.1365-2672.2008.03778.x

471 EC, 2005. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological
472 criteria for foodstuffs. Official Journal of the European Communitites 338, 1–26.

473 EFSA, 2018.*Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human
474 health in the EU. EFSA Journal 16, 1-5134.
475 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5134

476 Evans, E.W., Redmond, E.C., 2018. Behavioral observation and microbiological analysis of older
477 adult consumers' cross-contamination practices in a model domestic kitchen. Journal of Food
478 Protection 81, 569–581. https://doi.org/10.4315/0362-028X.JFP-17-378

479 Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H., 2017. Processed cheese and
480 substitute/imitation cheese products, in: Fundamentals of Cheese Science. Springer Nature,
481 New York, pp. 589–627.

482 Glass, K.A., Mu, M., LeVine, B., Rossi, F., 2017. Inhibition of *Clostridium botulinum* in model
483 reduced-sodium pasteurized prepared cheese products. Journal of Food Protection 80, 1478-
484 1488. https://doi.org/10.4315/0362-028X.JFP-17-027

485 Guinee, T.P., Products, D., Cork, C., 2004. Pasteurized processed cheese and substitute/imitation

cheese products, in: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P., (Eds.) Cheese: Chemistry, Physics and Microbiology. Elsevier Academic Press, London, pp. 349–394. https://doi.org/10.1016/S1874-558X(04)80052-6

Hereu, A., Dalgaard, P., Garriga, M., Aymerich, T., Bover-Cid, S., 2014. Analysis and modelling the growth behaviour of *Listeria monocytogenes* on RTE cooked meat products after a high pressure treatment at 400 MPa. International Journal of Food Microbiology, 186, 84-94. https: //doi: 10.1016/j.ijfoodmicro.2014.06.020

James, S.J., Evans, J., James, C., 2008. A review of the performance of domestic refrigerators. Journal of Food Engineering 87, 2–10. https://doi.org/10.1016/j.jfoodeng.2007.03.032

Kim, N.H., Lee, N.Y., Kim, M.G., Kim, H.W., Cho, T.J., Joo, I.S., Heo, E.J., Rhee, M.S., 2018. Microbiological criteria and ecology of commercially available processed cheeses according to the product specification and physicochemical characteristics. Food Research International 106, 468–474. https://doi.org/10.1016/j.foodres.2018.01.014

Le Marc, Y., Huchet, V., Bourgeois, C.M., Guyonnet, J.P., Mafart, P., Thuault, D., 2002. Modelling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. International Journal of Food Microbiology 73, 219–237. https://doi.org/10.1016/S0168-1605(01)00640-7

Loessner, M.J., Maier, S.K., Schiwek, P., Scherer, S., 1997. Long-chain polyphosphates inhibition growth of *Clostridium tyrobutyricum* in processed cheese spreads. Journal of Food Protection 60, 493–498. https://doi.org/10.4315/0362-028X-60.5.493

Maier, S.K., Scherer, S., Loessner, M.J., Maier, S.K., Scherer, S., 1999. Long-chain polyphosphate causes cell lysis and inhibits *Bacillus cereus* septum formation , which is dependent on divalent cations. Applied and Enviromental Microbiology 65, 3942–3949.

Martinez-Rios, V., Dalgaard, P., 2016. Modelling and predicting growth of psychrotolerant pseudomonads in milk and cottage cheese. International Journal of Food Microbiology 216, 110-120. https://doi.org/10.1016/j.ijfoodmicro.2015.09.020

Martinez-Rios, V., Gkogka, E., Dalgaard, P., 2019. New term to quantify the effect of temperature on pH_{min}-values used in cardinal parameter growth models for *Listeria monocytogenes*. Frontiers in Microbiology 10, 1510. https://doi.org/10.3389/fmicb.2019.01510

Maurer, A., 2012. Processed cheese manufacture and stabilization of dairy products. A JOHA® guide. BK Giulini GmbH: Ludwigshafen.

Mejlholm, O., Dalgaard, P., 2009. Development and validation of an extensive growth and growth boundary model for *Listeria monocytogenes* in lightly preserved and ready-to-eat shrimp. Journal of Food Protection 72, 2132–2143. https://doi.org/10.4315/0362-028X-72.10.2132

Mejlholm, O., Gunvig, A., Borggaard, C., Blom-Hanssen, J., Mellefont, L., Ross, T., Leroi, F., Else, T., Visser, D., Dalgaard, P., 2010. Predicting growth rates and growth boundary of *Listeria monocytogenes* - An international validation study with focus on processed and ready-to-eat meat and seafood. International Journal of Food Microbiology 141, 137–150. https://doi.org/10.1016/j.ijfoodmicro.2010.04.026

Mejlholm, O., Dalgaard, P., 2013. Development and validation of an extensive growth and growth boundary model for psychrotolerant *Lactobacillus* spp. in seafood and meat products. International Journal of Food Microbiology 167, 244–260. https://doi.org/10.1016/j.ijfoodmicro.2013.09.013

Mellefont, L.A., Ross, T., 2003. The effect of abrupt shifts in temperature on the lag phose duration of *Escherichia coli* and *Klebsiella oxytoca*. International Journal of Food Microbiology 60, 295-305. https://doi.org/10.1016/S0168-1605(02)00378-1

Oscar, T.P., 2007. Predictive models for growth of *Salmonella* typhimurium DT104 from low and high initial density on ground chicken with a natural microflora. Food Microbiology 24, 640-651. https://doi.org/10.1016/j.fm.2006.11.003

Redmond, E.C., Griffith, C.J., 2003. Consumer food handling in the home: a review of food safety studies. Journal of food protection 66, 130–61. https://doi.org/10.4315/0362-028X-66.1.130

Resnik, S.L., Chirife, J., 1988. Proposed theoretical water activity values at various tempertures for selected solutions to be used as reference sources in the range of microbial growth. Journal of Food Protection, 51, 419-423. https://doi.org/10.4315/0362-028X-51.5.419

Roccato, A., Uyttendaele, M., Membré, J.M., 2017. Analysis of domestic refrigerator temperatures and home storage time distributions for shelf-life studies and food safety risk assessment. Food Research International 96, 171–181. https://doi.org/10.1016/j.foodres.2017.02.017

Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. Journal of Applied Bacteriology 81, 501–508. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x

Ross, T., Dalgaard, P., 2004. Secondary models, in: Mckellar, R.C, Lu, X. (Ed.), Modeling Microbial Responses in Foods. CRC Press, Boca Raton, FL, USA., pp. 63–150.

Rosso, L., Bajard, S., Flandrois, J.P., Lahellec, C., Fournaud, J., Veit, P., 1996. Differential growth of *Listeria monocytogenes* at 4 and 8°C: Consequences for the shelf life of chilled products. Journal of Food Protection 59, 944–949. https://doi.org/10.4315/0362-028X-59.9.944

Schaffner, D.W., Ross, W.H., Montville, T.J., 1998. Analysis of the influence of enviromental parameters *Clostridium botulinum* time-to-toxicity by using three modeling approaches. Applied and Enviromental Microbiology, 64, 4416-4422.

ter Steeg, P.F., Cuppers, H.G.A.M., Hellemons, J.C., Rijke, G., 1995a. Growth of proteolytic *Clostridium botulinum* in process cheese products: I. Data acquisition for modeling the influence of pH, sodium chloride, emulsifying salts, fat, dry basis, and temperature. Journal of Food Protection 58, 1091–1099. https://doi.org/10.4315/0362-028X-58.10.1091

ter Steeg, P.F., Cuppers, H.G.A.M., 1995b. Growth of proteolytic *Clostridium botulinum* in process cheese products: II. Predictive modelling. Journal of Food Protection, 58, 1100-1108. https://doi.org/10.4315/0362-028X-58.10.1100

Tanaka, N., Trausman, E., Plantinga, P., Finn, L., Flom, W., Meske, L., Guggisberg, J., 1986. Evaluation of factors involved in antibotulinal properties of pasteurized process cheese spreads. Journal of Food Protection, 49, 526-531. https://doi.org/10.4315/0362-028X-49.7.526

564 Tompkin, R.B., 1983. Indirect antimicrobial effects in foods: phosphates. Journal of Food Safety 6,
565 13-27.

566 Velugoti, P.R., Bohra, L.K., Juneja, V.K., Huang, L., Wesseling, A.L., Subbiah, J., Thippareddi, H.,
567 2011. Dynamic model for predicting growth of *Salmonella* spp. in ground sterile pork. Food
568 Microbiology 28, 796–803. https://doi.org/10.1016/j.fm.2010.05.007

569 WHO, 2006. Five keys to safer food manual.
570 https://www.who.int/foodsafety/publications/5keysmanual/en/ (accessed 30 January 2019)

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Table 1. Product information for phosphate, citrate and commercial melting salts.

Abbreviation[a]	Name	Chemical compound name	Product number	Producer	E-number
P1	Orthophosphate	Sodium phosphate monobasic 2-hydrate	04269	Sigma-Aldrich	NA[b]
P2	Di-phosphate	Sodium pyrophosphate 10-hydrate	221368	Sigma-Aldrich	NA
P3	Tri-phosphate	Pentasodium tripolyphosphate	238503	Sigma-Aldrich	NA
TC	Trisodium citrate	Trisodium citrate	NA	BK Giulini GmbH	E331
DP	Disodium phosphate	Disodiumhydrogenphosphate-2 hydrate	NA	BK Giulini GmbH	E339ii
BUDAL	Budal® Na 322	Trisodium phosphate 12-hydrate	NA	Budenheim KG	E339iii
PZ35	JOHA® PZ35	Sodium orthophosphate, trisodium citrate	NA	BK Giulini GmbH	E339, E331
PZ6	JOHA® PZ6	Sodium orthophosphate, sodium diphosphate, sodium polyphosphates	NA	BK Giulini GmbH	E339, E450, E452
S9	JOHA® S9	Orthophosphates, polyphosphates	NA	BK Giulini GmbH	E339, E452
PZ189	JOHA® 189	Orthophosphates, polyphosphates, trisodium citrate	NA	BK Giulini GmbH	E339, E452, E331
S9K	JOHA® S9K	Potassium orthophosphates, potassium triphosphates	NA	BK Giulini GmbH	E340, E451

[a] Product name abbreviation used in the text
[b] NA: information not available

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Table 2. Storage conditions and product characteristics for challenge tests with processed spreadable cheese.

CT[b]	Batch	Type of cheese	n[c]	Storage temp. (°C)	Product characteristics (Avg.±SD)[a]							
					pH	a_w	Organic acids in water phase (ppm)			Melting salts in water phase (%)			
							Lactic acid	Acetic acid	Citric acid	P1[d]	P2[e]	P3[f]	TC[g]
1	1	Customized	3	14.9±0.2	6.1±0.1	0.972±0.000	9,970±2,013	1,158±53	1,337±293	2.61±NA[h]	<0.01	<0.01	0.23±NA
2	2	Customized	3	14.9±0.2	6.2±0.3	0.970±0.004	11,605±588	1,272±57	1,493±91	4.98±NA	<0.01	<0.01	0.25±NA
3	3	Customized	3	15.0±0.3	6.4±0.2	0.967±0.001	11,969±1,611	3,483±934	1,709±384	0.52±NA	1.62±NA	<0.01	0.25±NA
4	4	Customized	3	15.0±0.3	6.2±0.1	0.971±0.001	14,768±523	3,231±922	2,134±125	0.48±NA	5.09±NA	<0.01	0.26±NA
5	5	Customized	3	15.0±0.3	6.6±0.1	0.970±0.002	9,559±1,630	1,451±1,135	1,642±281	0.68±NA	0.49±NA	0.63±NA	0.23±NA
6	6	Customized	3	15.0±0.3	6.1±0.1	0.967±0.000	11,859±598	1,701±13	2,368±212	0.74±NA	1.45±NA	5.17±NA	0.28±NA
7	7	Customized	3	14.9±0.2	6.4±0.1	0.970±0.000	7,051±1,030	1,116±18	11,510±290	0.42±NA	<0.01	<0.01	2.78±NA
8	8	Customized	3	14.9±0.2	6.3±0.1	0.963±0.001	12,339±1,620	2,162±1,116	38,282±5,319	0.44±NA	<0.01	<0.01	5.01±NA
9	9	Customized	3	15.0±0.3	6.4±0.2	0.964±0.001	9,514±2,760	1,116±90	1,289±479	2.04±NA	0.34±NA	0.29±NA	0.27±NA
10	10	Customized	3	15.0±0.3	6.3±0.1	0.952±0.001	15,328±1,768	1,630±227	2,490±1,390	3.90±NA	0.54±NA	3.67±NA	0.28±NA
11	1	Commercial	3	22.0±0.2	6.2±0.0	0.969±0.001	6,371±22	958±4	518±12	1.90±NA	<0.01	<0.01	0.49±NA
12	2	Commercial	3	4.8±0.4	6.2±0.0	0.969±0.001	7,641±865	568±178	2,558±290	1.94±NA	<0.01	<0.01	0.50±NA
13	2	Commercial	3	10.1±0.2	6.2±0.0	0.969±0.001	7,641±865	568±178	2,558±290	1.94±NA	<0.01	<0.01	0.50±NA
14	2	Commercial	3	14.5±0.2	6.2±0.0	0.969±0.001	7,641±865	568±178	2,558±290	1.94±NA	<0.01	<0.01	0.50±NA
15	3	Commercial	3	4.8±0.4	6.2±0.0	0.972±0.001	13,105±4,612	1,559±345	5,392±1,826	1.91±NA	<0.01	<0.01	0.49±NA
16	3	Commercial	3	10.1±0.2	6.2±0.0	0.972±0.001	13,105±4,612	1,559±345	5,392±1,826	1.91±NA	<0.01	<0.01	0.49±NA
17	3	Commercial	3	14.5±0.2	6.2±0.0	0.972±0.001	13,105±4,612	1,559±345	5,392±1,826	1.91±NA	<0.01	<0.01	0.49±NA
18	4	Commercial	3	7.2±0.2	6.1±0.0	0.969±0.001	12,624±1,468	1,436±159	7,612±1,062	2.14±NA	<0.01	<0.01	0.57±NA
19	4	Commercial	3	11.1±0.2	6.1±0.0	0.969±0.001	12,633±763	1,594±140	7,708±402	2.13±NA	<0.01	<0.01	0.58±NA
20	4	Commercial	3	3.8-19.4[i]	6.3±0.0	0.975±0.000	8,368±717	1,042±226	5,416±284	1.92±NA	<0.01	<0.01	0.53±NA

[a] Avg.: average; SD: standard deviation (n = 3)
[b] Challenge test
[c] Number of growth curves per challenge test
[d] P1: orthophosphate
[e] P2: di-phosphate
[f] P3: tri-phosphate
[g] TC: trisodium citrate
[h] NA, not available as pooled sample was analysed by Eurofins.
[i] Dynamic storage temperature.

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Table 3. Growth parameters of *L. monocytogenes* in challenge tests with processed spreadable cheese.

CT[b]	Type of cheese	Growth parameter values (Avg. ±SD) [a]						ψ[c]	Predicted growth/no-growth responses
		Lag-time (h)	*RLT* (h)	Log N_0 (cfu/g)	Log N_{max} (cfu/g)	μ_{max} (1/h)	Duration of exp. (days)		
1	Customized	222±11	13.9±2.1	2.3±0.2	4.9±0.1	0.043±0.01	19.1	0.3	Correct
2	Customized	114±18	7.2±1.8	2.5±0.1	7.0±0.6	0.043±0.00	20.1	0.3	Correct
3	Customized	0.0±0.0[d]	0.0±0.0	2.4±0.1	2.2±0.1	0.000±0.00[e]	51.0	0.4	Fail-safe
4	Customized	0.0±0.0[d]	0.0±0.0	2.4±0.1	2.1±0.1	0.000±0.00[e]	65.1	1.5	Correct
5	Customized	0.0±0.0[d]	0.0±0.0	2.0±0.1	6.1±0.2	0.034±0.00	17.9	0.2	Correct
6	Customized	0.0±0.0[d]	0.0±0.0	2.4±0.1	1.3±0.3	0.000±0.00[e]	65.0	2.4	Correct
7	Customized	0.0±0.0[d]	0.0±0.0	2.4±0.2	7.7±0.2	0.102±0.00	8.0	0.1	Correct
8	Customized	0.0±0.0[d]	0.0±0.0	2.6±0.1	7.8±0.1	0.037±0.00	20.0	0.3	Correct
9	Customized	0.0±0.0[d]	0.0±0.0	2.2±0.2	2.4±0.2	0.000±0.00[e]	51.0	0.3	Fail-safe
10	Customized	0.0±0.0[d]	0.0±0.0	2.4±0.2	1.7±0.4	0.000±0.00[e]	65.0	1.2	Correct
11	Commercial	0.0±0.0[d]	0.0±0.0	2.0±0.1	7.8±0.0	0.106±0.00	17.9	0.2	Correct
12	Commercial	0.0±0.0[d]	0.0±0.0	1.4±0.1	3.4±0.3	0.006±0.00	32.0	0.3	Correct
13	Commercial	0.0±0.0[d]	0.0±0.0	1.3±0.2	7.2±0.2	0.016±0.00	45.1	0.2	Correct
14	Commercial	0.0±0.0[d]	0.0±0.0	1.3±0.0	7.0±0.1	0.051±0.00	16.2	0.2	Correct
15	Commercial	306±22	3.9±0.4	2.6±0.1	7.2±0.2	0.009±0.00	83.0	0.5	Correct
16	Commercial	43±16	1.6±0.6	2.9±0.0	7.8±0.1	0.027±0.00	29.8	0.4	Correct
17	Commercial	7±10[f]	0.6±0.9	2.7±0.1	7.9±0.2	0.056±0.00	17.0	0.3	Correct
18	Commercial	157±56	4.3±1.9	1.0±0.0	4.5±0.1	0.015±0.00	24.0	0.5	Correct
19	Commercial	0.0±0.0[d]	0.0±0.0	1.2±0.3	7.6±0.0	0.035±0.00	24.0	0.4	Correct
20	Commercial	-	-	1.9±0.2	7.6±0.3	-	24.0	-	

[a] Avg: average; SD: standard deviation
[b] Challenge test.
[c] ψ-value indicate how far the properties of a specific food product is from the predicted growth boundary of *L. monocytogenes* with ψ= 1.0.
[d] No significant lag-time
[e] No growth observed for duration of experiment.
[f] One growth curve had a significant lag time out of three growth curves.

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Table 4. Cardinal parameter values for the effect of melting salts on *L. monocytogenes* growth.

	Parameter values (value ±SE)[a]	n1	n2
MIC_{P1} (%)	14.9 ± 1.1	1	1
MIC_{P2} (%)	9.4 ± 0.4	1	2
MIC_{P3} (%)	7.6 ± 0.2	1	2
MIC_{TC} (%) or MIC_{CAC_U} (mM)	11.0 ± 0.3 or 0.75 ± 0.02	1	1

[a] SE: standard error

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Table 5. Observed and predicted effect of commercial melting salt preparations for growth of *L. monocytogenes*.

Commercial melting salts	Conc. studied (g/ml)	Percentage composition				n[a]	B_f[b]	A_f[c]
		P1	P2	P3	TC			
DP	0.0-9.1	55	0	0	0	12	1.32	1.47
BUDAL	0.0-15.7	26	0	0	0	8	2.09	2.26
PZ35	0.0-8.2	15	0	51	0	18	1.24	1.24
PZ6	0.0-3.9	3	15	6	11	24	1.52	1.53
S9	0.0-3.4	4	5	15	0	14	1.53	1.53
PZ189	0.0-4.5	25	0	0	32	8	1.34	1.35
S9K	0.0-3.5	26	2	28	0	10	1.16	1.18
All data						94	1.42	1.46

[a] n, number of experiments
[b] B_f, bias factor
[c] A_f, accuracy factor

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Table 6. Comparison of observed and predicted growth of *L. monocytogenes* in spreadable processed cheese by bias and accuracy factors.

	B_f[a]	A_f[b]
New model[c] using MIC_{TC} (%)	1.06	1.35
New model[c] using MIC_{CAC_U} (mM)	1.12	1.29
Modified Mejlholm & Dalgaard (2009) model[d]	1.15	1.34
Original Mejlholm and Dalgaard (2009) model[e]	1.70	1.82
Augustin et al. (2005), cheese[e]	0.93	1.30
ComBase[e]	2.65	2.65

[a] B_f, bias factor where $0.95 < B_f < 1.11$ indicates a good model performance, with B_f 1.11-1.43 or 0.87-0.95 corresponding to acceptable model performance and B_f <0.87 or >1.43 reflecting unacceptable model performance

[b] A_f, accuracy factor with $A_f > 1.5$ suggested to indicate an incomplete model or systematic deviation between observed and predicted μmax-values

[c] Predicted by the new model (eq. (2) through eq. (5))

[d] Predicted by the Mejlholm and Dalgaard (2009) model using its $MIC_{U\,CAC}$-value (2.21 mM) but expanded with MIC-terms for phosphate salts (P1, P2, P3) as determined in the present study

[e] Growth and no-growth prediction responses were 74% correct with 26% fail-safe and 0% fail-dangerous

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Table 7. Comparison of predicted and observed growth data using the acceptable simulation zone (ASZ) method.

CT[a]	Storage temperature (°C) and growth data	% observations within ASZ[d] Predictions performed with new model including $MIC_{U\ CAC}$ (mM)
1[c]	14.9±0.2, Fig 3a	17 (22/56)
2[c]	14.9±0.2	32 (45/41)
5[c]	15.0±0.3	41 (46/26)
7[c]	14.9±0.2	67 (48/15)
8[c]	14.9±0.2, Fig.3b	95 (79/13)
11[b]	22.0±0.2, Fig. 3c	97 (93/43)
12[b]	4.8±0.4	62 (64/44)
13[b]	10.1±0.2	32 (32/49)
14[b]	14.5±0.2	40 (30/30)
15[b]	4.8±0.4, Fig. 3d	31 (70/33)
16[b]	10.1±0.2, Fig. 3e	98 (100/21)
17[b]	14.5±0.2, Fig. 3f	100 (70/18)
18[b]	7.2±0.2, Fig. 3g	88 (88/54)
19[b]	11.1±0.2	67 (45/27)
20[b]	Dynamic (3.8-19.4°C), Fig. 3h	91 (21/18)
	Average ASZ score	
All data		58 (57/33)
Commercial		63 (61/34)
Customized		49 (49/31)

[a] Challenge test
[b] Customized spreadable processed cheese
[c] Commercial spreadable processed cheese
[d] Calculation of ASZ score with minimum RLT-value (average/maximum RLT-value).

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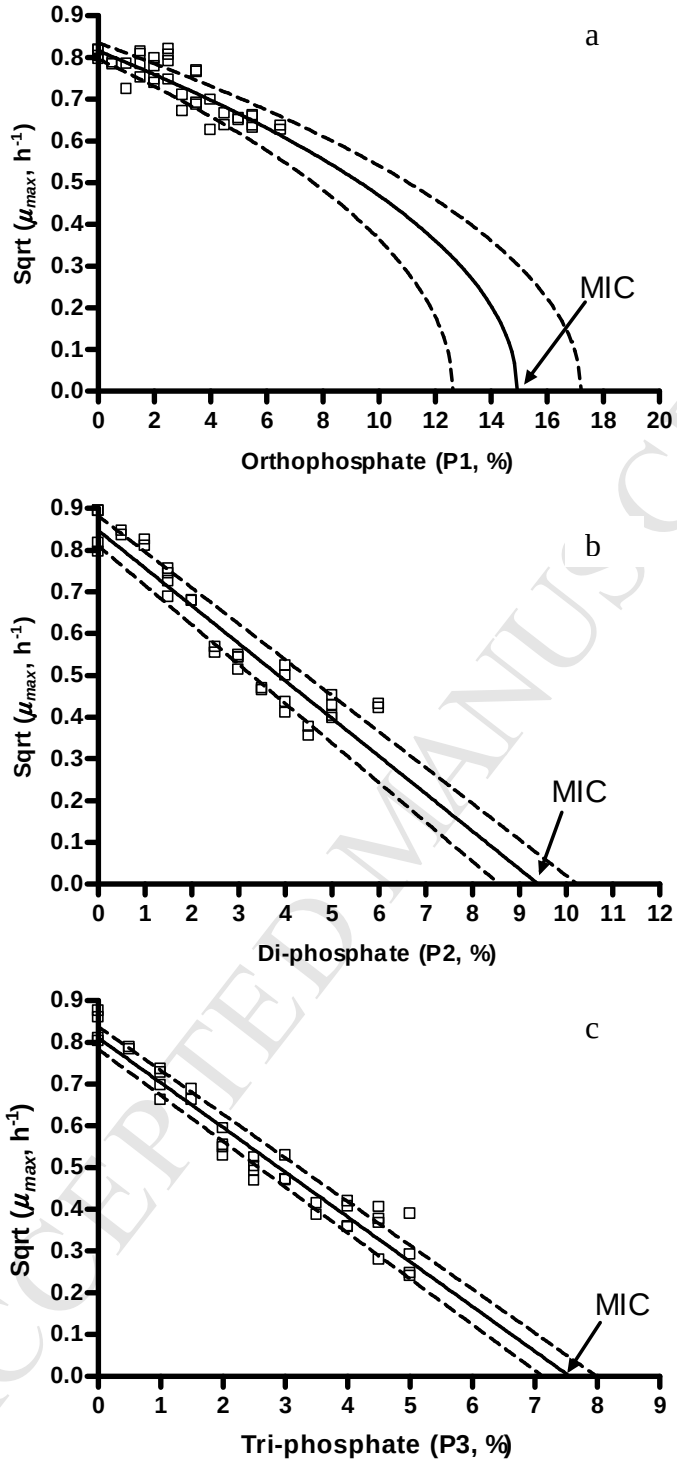

Fig.1. Maximum specific growth rates (μ_{max}, 1/h) of *L. monocytogenes* in BHI broth at 25°C and pH 6.2 as influenced by increasing concentrations of orthophosphate (a; P1), di-phosphate (b; P2) and tri-phosphate (c; P3). MIC-values for phosphate salts were determined by fitting eq. (3) to observed data (□). Solid and dashed lines represent the fitted (eq.(3)) and confidence intervals (95%), respectively.

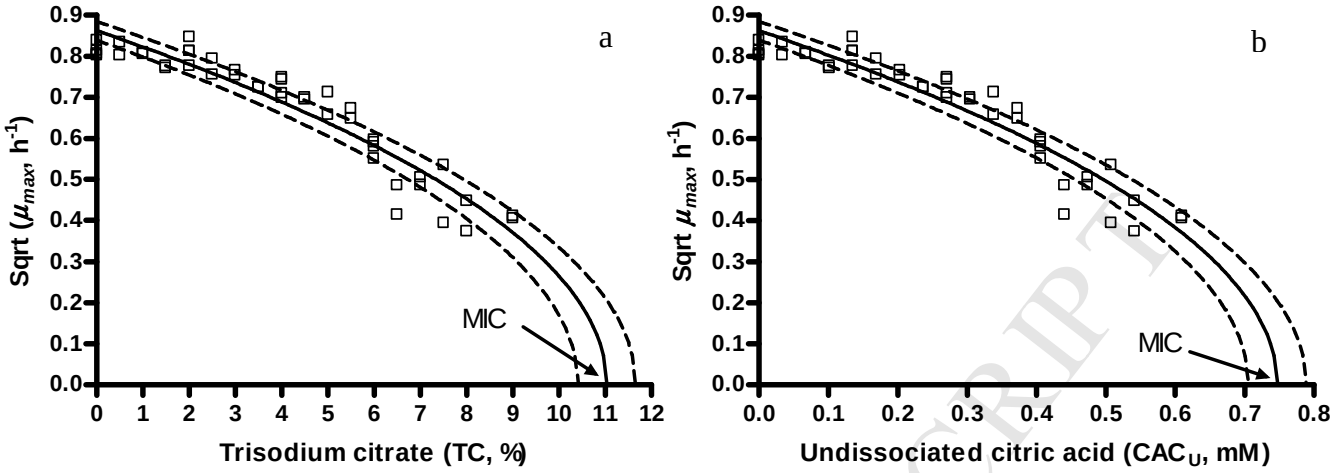

Fig.2. Effect of trisodium citrate (TC) (a) or undissociated citric acid (b) on maximum specific growth rates (μ_{max}, 1/h) of *L. monocytogenes* in BHI broth at 25°C and pH 6.2. MIC-values of citrate salts and undissociated citric acid were determined by fitting eq. (3) to observed data (□). Solid and dashed lines represent the fitted (eq. (3)) and confidence intervals (95%), respectively.

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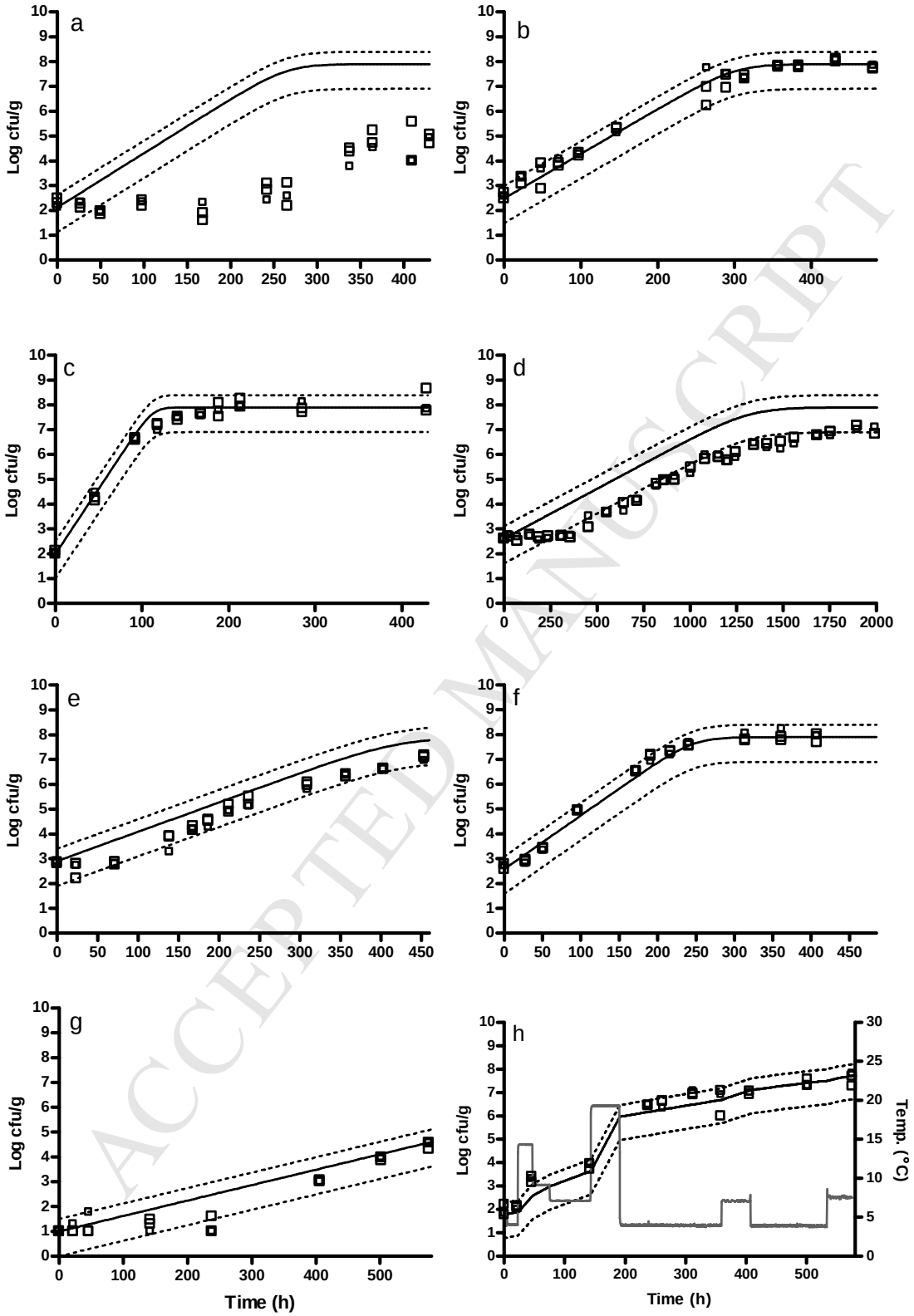

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Fig.3. Comparison of observed (□) and predicted (-) growth of *L. monocytogenes*. Spreadable processed spread cheese was studied at 22.0±0.2°C (a), 4.8±0.4°C (b), 10.1±0.2°C (c), 14.5±0.2°C (d), 14.9±0.2°C (e), 14.9±0.2 (f), 7.2±0.2 (g) and dynamic storage temperature 3.8-19.4°C (h, temperature profile is shown as grey lines). Solid lines represent the predicted growth by eq. (4) when using MIC_{CAC_U} (mM). Graphs include the ASZ (+0.5 and -1.0 Log cfu/g, dashed lines).

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Highlights

- Model to predict growth of *Listeria monocytogenes* in spreadable processed cheese
- Model including the effect of nine environmental factors
- Support tool for product development/reformulation of spreadable processed cheese

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Conflict of interest: Elissavet Gkogka is employed by Arla Foods

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