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A model to predict the fate of *Listeria monocytogenes* in different cheese types – A major role for undissociated lactic acid in addition to pH, water activity, and temperature

E. Wemmenhove[a,b,1], M.H.J. Wells-Bennik[a,*], M.H. Zwietering[b,*]

[a] NIZO, Ede, the Netherlands
[b] Food Microbiology, Wageningen University, the Netherlands

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ABSTRACT

Undissociated lactic acid has been shown to play a major role in complete growth inhibition of *Listeria monocytogenes* in Gouda cheese. In addition, low water activity conditions may contribute to growth inhibition. In the current study, it was assessed whether the major factors that inhibit growth of *L. monocytogenes* in Gouda cheese are the factors that determine growth in other types of ready-to-eat cheese as well.

Various types of cheeses were selected, some of which had been associated with listeriosis, while others had not. Based on the composition of the different cheese types, the concentrations of undissociated lactic acid were calculated for each type. The ability to support growth of *L. monocytogenes* was predicted using the Gamma model, based on literature data on total lactic acid content, moisture content, fat content, pH, Aw, and temperature, and optimal growth rates in milk at 30–37 °C. In addition, the actual specific growth rates of *L. monocytogenes* in the various cheeses were calculated based on available experimental growth data. In 9 out of the 10 RTE cheeses reviewed, the undissociated lactic acid concentrations and a_w determined growth/no growth of *L. monocytogenes*. No growth was correctly predicted for feta, Cheddar and Gouda, and growth was correctly predicted for ricotta, queso fresco, Camembert, high-moisture mozzarella, cottage and blue cheese. Growth of *L. monocytogenes* was not observed in practice upon inoculation of Emmental, whereas growth in this cheese type was predicted when including the above mentioned factors in the models. Other factors, presumably acetic and propionic acid, are thought to be important to inhibit growth of the pathogen in Emmental. The results from our study show that for cheeses in which lactic acid is a main acid, our model based on undissociated lactic acid, temperature, pH and a_w gives a good prediction of potential outgrowth of *L. monocytogenes*. Implications for *L. monocytogenes* legislation are discussed per type of RTE cheese reviewed.

1. Introduction

Most cheeses are intended to be ready-to-eat (RTE) products and do not undergo a heating step prior to consumption. RTE cheeses can be manufactured from pasteurized milk or from raw milk. Various RTE cheeses, such as Latin-style, blue-veined, and mould cheeses have been linked to cases of listeriosis (Jackson et al., 2018), a disease caused by *Listeria monocytogenes* (Swaminathan and Gerner-Smidt, 2007). *L. monocytogenes* may be present at low concentrations in raw milk (Table 1) and RTE cheeses manufactured from raw milk may therefore pose a risk (Jackson et al., 2018). It is unlikely that *L. monocytogenes* survives pasteurization of the cheese milk (den Besten and Zwietering, 2012). Yet, this pathogen can survive in highly acidic, salty and low-temperature environments (ICMSF, 1996), it can form biofilms (Borucki et al., 2003; Cossart et al., 2003; Carpentier and Cerf, 2011) and it may be persistent in the RTE cheese processing environment. Contamination of finished products must be prevented, especially as the product does not undergo a heat treatment prior to consumption.

EU regulation EC 2073/2005 lays down certain process hygiene criteria to indicate the correct functioning of the production process (European Commission, 2005). The regulation also lays down food safety criteria for relevant foodborne bacteria, their toxins and metabolites in specific foods. The given criteria define the acceptability of a product placed on the market. Specific criteria for *L. monocytogenes*

* Corresponding authors.
E-mail addresses: marjon.wells-bennik@nizo.com (M.H.J. Wells-Bennik), marcel.zwietering@wur.nl (M.H. Zwietering).
1 Current affiliation: Arla Foods Ingredients, Videbæk, Denmark

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Table 1
Prevalence of *L. monocytogenes* in raw milk based on presence/absence of *L. monocytogenes* detected in 25 g or ml.

Total samples (n)	Amount of positive samples	Prevalence (%)	Country of origin	References
137	6	4.4	The Netherlands	Beckers et al. (1987)
176	27	15.3	Northern Ireland	Harvey and Gilmour (1992)
589	29	4.9	Ireland	Rea et al. (1992)
774	28	3.6	Spain	Gaya et al. (1998)
295	58	19.7	Sweden	Waak et al. (2002)
143	9	6.3	Belgium	de Reu et al. (2004)
24	1	4.2	Ireland	Fox et al. (2009)
230	0	0	Austria	Schoder et al. (2011)
297	2	0.7	New Zealand	Hill et al. (2012)
446	97	21.7	Iran	Jamali et al. (2013)
468	11	2.4	France	Meyer-Broseta et al. (2003)
182	10	5.5	Finland	Ruusunen et al. (2013)
3761	278	7.4	Total	–

apply to RTE foods, including RTE cheeses. Under all circumstances, it is important to avoid potential contamination of the product with this pathogen. This relates to ingredients and post-processing contamination. Products other than those intended for infants and for special medical purposes are categorized based on the ability of the food product to support (category 1.2) or not support (category 1.3) growth of *L. monocytogenes*. Food products with pH $\leq$ 4.4 or with $a_w \leq 0.92$ and food products with pH < 5.0 and $a_w \leq 0.94$ are automatically considered as a food product belonging to category 1.3. Other food products may also belong to this category, subject to scientific evidence. An overview of the European food safety criteria for *L. monocytogenes* in RTE foods is presented in Fig. 1, including the sampling plan and stage at which each criterion applies per category. The US has a zero-tolerance approach for all RTE foods, with absence in 25 g; the FDA states that absence in 25 g needs to be demonstrated in two 25 g samples compiled from two 125 g samples (FDA). While a 'zero-tolerance' applies to RTE foods in the USA, it may be valuable to know whether the product supports growth or not, as part of a risk based approach (Farber et al., 2021).

Various authorities have classified different cheese types according to their potential risk related to listeriosis. The classification of cheese by the Food and Drug Administration (FDA) in the USA is based on the moisture on whole cheese content (moisture%) (FDA, 2003). Based on cluster analysis of predicted per serving and per annum relative ranking, consumption of soft unripened cheese (e.g. cottage, ricotta) is considered of high risk, soft ripened cheese (brie, Camembert, feta, mozzarella) and semi-soft cheese (blue) of moderate risk, and hard cheese (Cheddar, parmesan) of very low risk for listeriosis.

Listeriosis cases have never been linked with Gouda cheeses made from pasteurized milk. Gouda cheese, having a moisture% of 39 to 53% (van den Berg et al., 2004) is classified as a semi-soft cheese according to the FDA definition. With a pH > 5.0 and $a_w > 0.94$, Gouda is not automatically classified as a cheese that does not support growth of *L. monocytogenes* based on criteria mentioned in EC 2073/2005 (European Commission, 2005). For Gouda cheese, it was demonstrated by challenge tests that it does not support growth of *L. monocytogenes* (Northolt et al., 1988; Wemmenhove et al., 2013 and 2014). Undissociated lactic acid was established as the key parameter inhibiting this growth during initial ripening, together with a_w and pH (Wemmenhove et al., 2016a and 2018). Upon prolonged ripening periods and in cheese rind, low water activity conditions can lead to full growth inhibition of *L. monocytogenes* (Wemmenhove et al., 2016b).

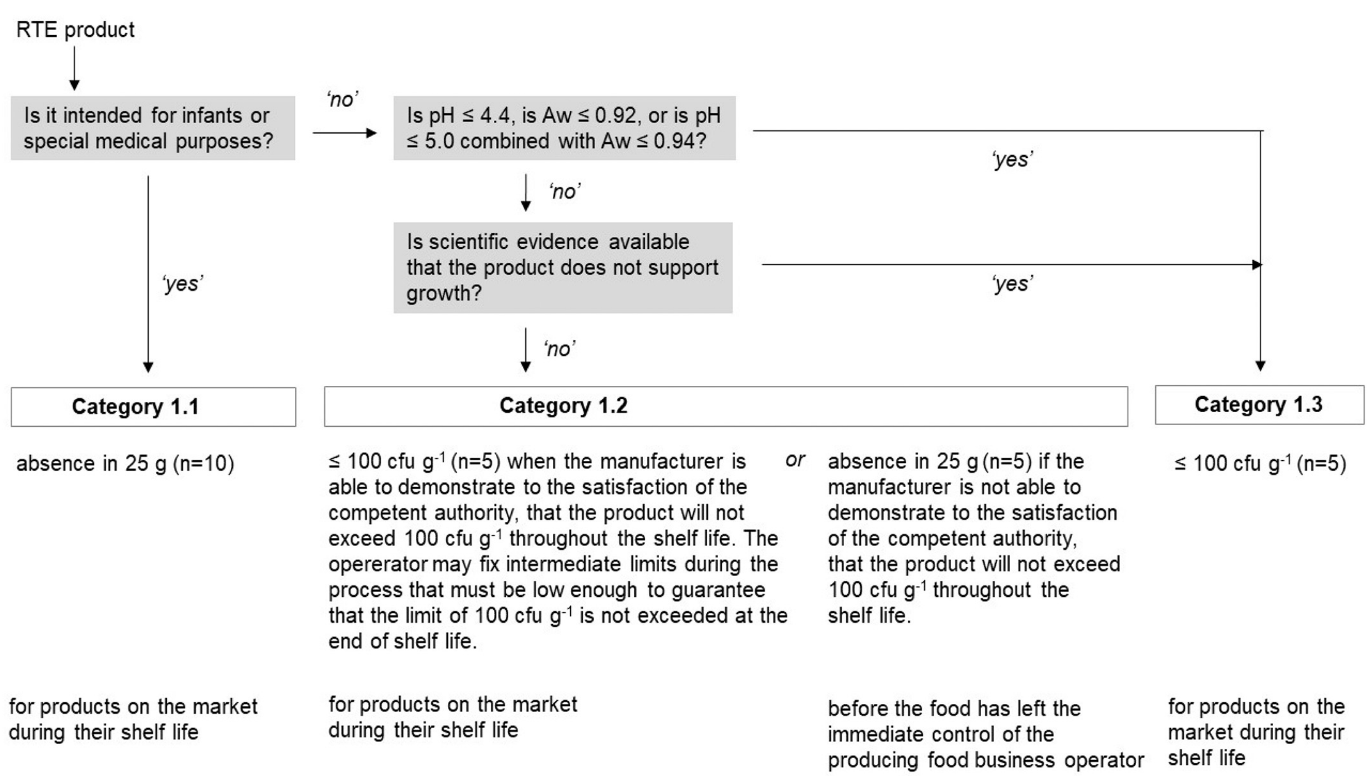

Fig. 1. Overview of the European food safety criteria for *L. monocytogenes* in RTE foods defined in EC 2073. Below each category, the sampling plan and the stage at which each criterion applies are shown.

The aim of this study was to establish whether undissociated lactic acid, together with pH and a_w also determine the fate of *L. monocytogenes* in cheeses other than Gouda. A literature review was performed to investigate associations of certain cheeses with listeriosis cases or absence thereof. Based on this overview, four cheese types were identified with links to listeriosis cases, while for six cheeses no links were established. Based on the compositions of Gouda cheese and nine other cheeses (i.e., blue, Camembert, Cheddar, cottage, Emmental, feta, high-moisture mozzarella, queso fresco, ricotta), undissociated lactic acid concentrations were calculated and growth / no growth predictions were made based on the Gamma model for these 10 cheeses at a reference temperature of 10 °C. In addition, actual specific growth rates in these other cheeses were calculated by extracting growth data based on published experimental studies and adjusted to the same reference temperature. These growth rates, based on practical observations, were subsequently compared with the growth rates as calculated with the model, to verify the predictions.

2. Materials and methods

2.1. Physicochemical properties of cheeses

Data on physicochemical properties (e.g. total lactic acid content, moisture content, fat content, pH, a_W) and storage temperature were obtained for 10 cheeses, namely, blue, Camembert, Cheddar, cottage, Emmental, feta, Gouda, mozzarella (high-moisture), queso fresco, and ricotta. Data were extracted from cheese handbooks and scientific literature (Supplementary Table A.1). The concentration of undissociated lactic acid in the different cheeses was calculated using Eq. 6.12 from Wemmenhove et al. (2018).

2.2. Prediction of specific growth rates based on cheese composition

Optimal growth rates (μ_{opt}) for *L. monocytogenes* were extracted from Combase based on specific growth rates in milk at 30–37 °C; the values used were 1.69 h^{-1} (maximum value) and 0.73 h^{-1} (average value). Specific growth rates μ in cheese were predicted using the Gamma model of Zwietering et al. (1992), a 'worst-case' model as it does not include interaction between factors. The Gamma factors for temperature, pH and water activity were calculated using $\gamma_T = \left(\frac{T-Tmin}{Topt-Tmin}\right)^2$, $\gamma_{pH} = \frac{(pH-pHmin)\cdot(2\cdot pHopt-pHmin-pH)}{(pHopt-pHmin)^2}$ and $\gamma_{a_w} = \frac{a_w-a_w min}{1-a_w min}$ according to Zwietering et al. (1996), with $T_{min} = -1.5$ °C, $T_{ref} = 10$ °C, $T_{opt} = 37$ °C, $pH_{min} = 4.4$, $pH_{opt} = 7.0$, $a_{wmin} = 0.92$ according to Te Giffel and Zwietering et al. (1996). The Gamma factor of undissociated lactic acid for *L. monocytogenes* was calculated using $\gamma_{HLac} = 1 - \frac{[HLac]}{6.35}$ according to Le Marc et al. (2002), with HLac as the concentration of undissociated lactic acid in mM and with a Minimal Inhibitory Concentration (MIC) of 6.35 mM as maximum MIC reported in literature (Aryani et al., 2015).

2.3. Actual specific growth rates of L. monocytogenes in different cheeses based on reported growth

Actual specific growth rates μ of *L. monocytogenes* in different cheeses were calculated based on data available in published challenge studies. Data were obtained through a literature search (searching Scopus and Web of Science for: monocytogenes AND cheese AND (Blue OR Camembert OR Cheddar OR Cottage OR Emmental OR Feta OR Gouda OR Mozzarella OR Queso Fresco OR Ricotta OR Swiss), sorted on relevance) and Combase (search on www.combase.cc for *Listeria monocytogenes* AND cheese, data from all scientific article records were incorporated for each of the 10 cheese types).

The $\mu_{10\ °C}$ values were calculated for each type of cheese using $\frac{\Delta logN \cdot ln(10)}{t}$, with Δlog N as the log concentration of *L. monocytogenes* at the end of sampling minus the log concentration after pressing, and with t as the time between the end of sampling and after pressing. The predicted specific growth rates ($\mu_{10\ °C}$ values) were extracted from Combase (www.combase.cc) when searching for growth rates of *L. monocytogenes* in milk at 30–37 °C. The $\mu_{10\ °C}$ values were based on Gamma factors for undissociated lactic acid, a_w, temperature and pH calculated according to Zwietering et al. (1996) and Te Giffel and Zwietering (1999), and a MIC of undissociated lactic acid for *L. monocytogenes* of 6.35 mM according to Aryani et al. (2015). Average conditions were chosen for undissociated lactic acid, a_w and pH (according to Table 2). The temperature was normalized to 10 °C by use of the square-root function of McMeekin et al. (1993): $\mu_{ref} = \mu_T \cdot \frac{(T_{ref}-T_{min})^2}{(T-T_{min})^2}$, with $T_{min} = -1.5$ °C and $T_{ref} = 10$ °C and μ_T as the specific growth rate at temperature T. The application of the square-root function by McMeekin et al. (1993) for temperature is only validated for positive actual specific growth rates. Similar to the positive actual specific growth rates, the negative growth rates were also normalized to 10 °C, using this function.

2.4. Cheeses associated with listeriosis

An overview was made of cheeses actually linked with listeriosis. Data were obtained through a literature search (searching Google, EFSA reports, CDC reports, Scopus and Web of Science for: listeriosis AND cheese, or 'Listeria', 'outbreak' and 'cheese', sorted on relevance, first 300 hits) for the years 1983–2019.

3. Results

3.1. Undissociated lactate and a_w of 10 types of cheeses

The values of undissociated lactic acid, pH, a_w and storage temperatures of 10 different cheese types, namely, blue, Camembert, Cheddar, cottage, Emmental, feta, Gouda, mozzarella (high-moisture), queso fresco, and ricotta, are listed in Supplementary Table A.1 and the average values are presented in Table 2. These data show that high concentrations of undissociated lactic acid are found in Cheddar, feta and Gouda, even higher than the MIC of 6.35 mM required to fully inhibit growth of *L. monocytogenes* (Aryani et al., 2015). Cottage, queso fresco, and ricotta contain very low concentrations of undissociated lactic acid and the a_w of the latter cheeses is high. In blue cheese, the concentration of undissociated lactic acid is <6.35 mM, but the a_w value (0.925) approximates the growth limit of *L. monocytogenes*. In Camembert, high-moisture mozzarella, and Emmental, the average concentration of undissociated lactic acid is 1.01, 3.09 and 2.80 mM and the a_w is 0.965, 0.940 and 0.970, but these values do not approximate the growth limits of *L. monocytogenes*.

3.2. Prediction of growth of L. monocytogenes in 10 types of cheeses

In addition to physicochemical properties, Table 2 displays predicted specific growth rates of *L. monocytogenes* per cheese type, which are calculated based on the physicochemical properties of the various cheese types and optimal growth rates in milk of *L. monocytogenes*, as obtained from Combase which contains an overview of studies on growth of *L. monocytogenes* in milk. For Cheddar, feta and Gouda, full growth inhibition is predicted (based on undissociated lactic acid). The highest growth of *L. monocytogenes* is predicted for ricotta and queso fresco, and some growth is predicted in blue, Camembert, high-moisture mozzarella and Emmental.

3.3. Growth of L. monocytogenes in cheeses based on published challenge studies

Supplementary Table A.2 displays specific growth rates of *L. monocytogenes* in and on 10 cheese types, as extracted from challenge

Table 2
Average values for total lactic acid content, moisture content, fat content, pH, a_w, temperature (T), the calculated undissociated lactic acid and the resulting Gamma factors, calculated from these average values and from the formulas in Supplementary Table A.1 according to paragraph 2.2 of the Materials and Methods section.

Type of cheese	Cheese category, as grouped by FDA (2003)	Total lactic acid content	Moisture content	Fat content	pH		a_w		T		Undissociated lactic acid		μ (h^{-1}) calculated based on μ_{opt} = 1.69 h^{-1}	μ (h^{-1}) calculated based on μ_{opt} = 0.73 h^{-1}	$\mu_{10°C}$ (h^{-1}) calculated based on μ_{opt} = 1.69 h^{-1}	$\mu_{10°C}$ (h^{-1}) calculated based on μ_{opt} = 0.73 h^{-1}
		Value (g per kg cheese)	Value (g per 100 g cheese)	Value (g per 100 g cheese)	Value	*Gamma factor*	Value	*Gamma factor*	Value (°C)	*Gamma factor*	Value (mM)	*Gamma factor*				
Blue	Semi-soft	10.8	40.5	32.6	5.93	*0.83*	0.925	*0.06*	11.5	*0.11*	1.7	*0.72*	0.0073	0.0031	0.0057	0.0025
Camembert	Soft ripened	10.0	48.5	23.7	6.06	*0.87*	0.965	*0.56*	8	*0.06*	1.01	*0.84*	0.042	0.018	0.062	0.027
Cheddar	Hard	15.0	37.5	34.4	5.2	*0.52*	0.95	*0.37*	10	*0.09*	13.9	*0*	0	0	0	0
Cottage	Soft unripened	0.29	79.5	3.9	4.85	*0.31*	0.99	*0.88*	17	*0.23*	0.27	*0.96*	0.102	0.044	0.040	0.017
Feta	Soft ripened	14.0	53.5	20.2	4.65	*0.18*	0.96	*0.50*	5.5	*0.03*	29.8	*0*	0	0	0	0
Gouda	Semi-soft	14.7	38.5	31.0	5.25	*0.55*	0.945	*0.31*	12.5	*0.13*	11.8	*0*	0	0	0	0
High-moisture mozzarella	Soft ripened	8.25	47.2	21.0	5.5	*0.67*	0.94	*0.25*	4	*0.02*	3.09	*0.51*	0.0030	0.0013	0.013	0.0056
Queso Fresco	Fresh soft	1.0	48.6	19.0	6.36	*0.94*	0.985	*0.81*	10	*0.09*	0.051	*0.99*	0.11	0.049	0.11	0.049
Ricotta	Soft unripened	1.93	63.2	30.5	6.32	*0.93*	0.996	*0.95*	11	*0.11*	0.083	*0.99*	0.16	0.067	0.13	0.057
Emmental	Hard	9.0	36.7	29.7	5.7	*0.75*	0.97	*0.62*	14	*0.16*	2.8	*0.57*	0.073	0.031	0.040	0.017

The Gamma factors are displayed in italics. In addition to the predicted specific growth rate μ, the growth rate $\mu_{10°C}$ was calculated as described in paragraph 2.3.

studies that have been published previously. The reported challenge studies were performed at different storage temperatures. As low storage temperature in itself may be a growth-limiting factor, results of the different challenge studies cannot be compared directly. To adjust for the effect of temperature on growth inhibition of *L. monocytogenes*, the challenge data were normalized to a storage temperature of 10 °C. Fig. 2 shows the actual and predicted specific growth rates whilst normalizing to a storage temperature of 10 °C. In the cheeses blue, Camembert, cottage, high-moisture mozzarella, queso fresco, ricotta and Emmental, growth of *L. monocytogenes* was predicted, which is in line with results from challenge tests (Supplementary Table A.2), except for Emmental for which growth was only predicted but not observed. In feta and Gouda, no growth of *L. monocytogenes* was observed nor predicted. In Cheddar, no growth was predicted and in most cases growth was not observed in most reported challenge tests. Two data points showed growth, but in these cases, no information on cheese characteristics were given (see discussion section). Overall, correct prediction of growth or no growth of *L. monocytogenes* was obtained for 9 out of 10 cheese types.

3.4. Overview of listeriosis outbreaks associated with cheese

Table 3 consists of an overview of listeriosis outbreaks that were linked with RTE cheeses. Mainly soft cheeses (e.g. queso fresco, ricotta, mould-ripened cheese) have been linked with listeriosis. Those soft cheeses were all able to support growth of *L. monocytogenes*, according to challenge studies published previously (Fig. 2). For the hard cheeses linked with listeriosis (mature cheese, Pave du Nord), it was not reported whether the cheese milk was raw or pasteurized.

4. Discussion

Similar to Gouda cheese, the same four factors (i.e., undissociated lactic acid, temperature, pH and a_w) were found to be important for growth inhibition of *L. monocytogenes* in/on other types of RTE cheeses. For 9 out of 10 cheese types, correct predictions of growth / no growth were obtained when including the factors undissociated lactic acid, temperature, pH and a_w in the prediction. Outgrowth of *L. monocytogenes* was correctly predicted for ricotta, queso fresco, Camembert, high-moisture mozzarella, cottage and blue). No growth was correctly predicted for feta, Cheddar and Gouda, except for two data points based on one study by Cui et al. (2016) who reported growth of *L. monocytogenes* on the surface of commercial Cheddar cheese; in that study, however, acid content, pH, and salt content were not specified, nor did they report on possible growth of moulds, which may lead to a rise in pH and consequently a reduction in concentrations of undissociated acid. Our results show the importance of undissociated lactic acid, temperature, pH and a_w for cheese in general.

In Fig. 2, our predictions are indicated with an arrow. More elaborate predictions of the growth rate were not performed, as undissociated lactic acid is a critical model factor, but the amount of obtained data on total lactic acid content was limited for many of the studied cheeses (Supplementary Table A.1). Per cheese type analyzed, there is a large variation in the actual specific growth rates. Part of the variation could be caused by physicochemical properties of the cheese that may differ between productions and may alter during cheese production and storage (Supplementary Table A.1). Another part of the variation could be due to variation in properties of *L. monocytogenes* strains that were used in the challenge tests (e.g. variation in growth rates results from Combase data, and acid sensitivity as described by Wemmenhove et al. (2016a)).

The model predictions are based on Gamma factors for undissociated lactic acid, pH, a_w and temperature. The Gamma factor for undissociated lactic acid was calculated based on values extracted from literature for total lactic acid content, moisture content, fat content and pH. Fat in a cheese with equal dry matter only minimally affects the concentration of undissociated lactic acid, as can be deduced from Equation [6.12] in

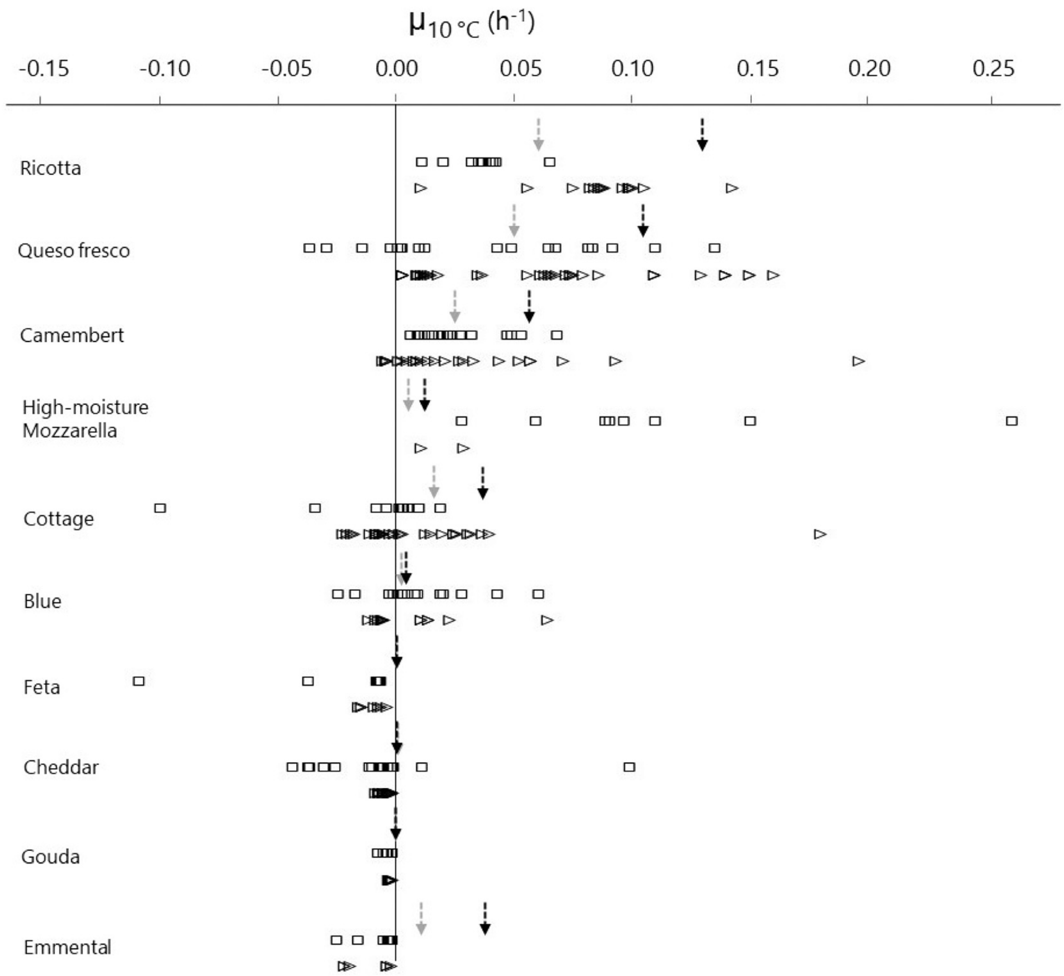

Fig. 2. Comparison of the actual and predicted specific growth rates of *L. monocytogenes* for 10 different RTE cheese types at 10 °C, to validate the importance of the factors undissociated lactic acid, a_w and pH on growth inhibition. The actual growth rates of *L. monocytogenes* were extracted from 58 challenge studies ($n = 308$ for 10 cheese types, data described in Supplementary Table A.2) and were compared to predicted growth rates (Table 2). Per cheese type, the squares display the growth rates for *L. monocytogenes* after artificial contamination of the surface or crust, and the triangles display the growth after artificial contamination of the cheese curd or milk. The $\mu_{10\,°C}$ values calculated with $\mu_{opt} = 1.69\ h^{-1}$ (maximum) are indicated with black arrows, and those calculated with $\mu_{opt} = 0.73\ h^{-1}$ (average) are indicated with grey arrows. A detailed description of the calculation of the actual and predicted specific growth rates is displayed in paragraphs 2.2 and 2.3 of the Materials and Methods section.

Wemmenhove et al. (2018). This minimal effect of fat content on the fate of *L. monocytogenes* was confirmed by an additional study, showing that the fate of *L. monocytogenes* in model Gouda cheeses with a normal fat content of 48% *w*/w fat in dry matter was the same as that of a low fat content of 30% w/w fat in dry matter that had a similar acidification curve (results not shown). Leong et al. (2014) and Mehta and Tatini (1994) also did not report differences in the fate of *L. monocytogenes* in normal-fat versus low-fat cheese. Less inhibition of growth of *L. monocytogenes* is only expected in low-fat cheese with an increased moisture content, because such cheese will have a lower concentration of undissociated lactic acid (Wemmenhove et al., 2018) and the a_w will be higher (Wemmenhove et al., 2014). In low-salt cheese, the a_w is increased and this may result in less growth inhibition of *L. monocytogenes* due to a_w. In most cheeses analyzed in Table 2, a_w is not the primary inhibiting factor. In blue cheese, however, a_w is the primary growth-inhibiting factor; for such a cheese, lowering of the salt content may result in less growth inhibition.

The factors undissociated lactic acid, temperature, pH and a_w do not fully explain why growth of *L. monocytogenes* is inhibited in practice upon inoculation in cheese milk used to make Emmental or inoculation on the surface of Emmental. In the challenge studies of Buazzi et al. (1992) and Bachmann and Spahr (1995), *L. monocytogenes* was inoculated into the milk, that was then curded and subjected to 2 min at 65 °C and 45 min at 53 °C. In the other challenge studies with Emmental (Genigeorgis et al., 1991; Leong et al., 2014), *L. monocytogenes* did not undergo a heating process, but the pathogen was inactivated. Acetic acid, propionic acid and free fatty acids are anticipated to be important factors for inhibition of growth of *L. monocytogenes* in Emmental, in addition to storage temperature, pH and a_w. Inhibition of growth of *L. monocytogenes* by acetic and propionic acid has been described (Wemmenhove et al., 2016a), and inhibition of *L. monocytogenes* by free fatty acids has also been reported (Wemmenhove et al., 2018). Currently, no model is available to predict growth in cheese based on undissociated acetic acid and propionic acid, or combinations thereof, or of these acids in combination with undissociated lactic acid in cheese.

The actual specific growth rates (normalized at 10 °C) of *L. monocytogenes* in and on high-moisture mozzarella that have been reported are considerably higher than the predicted growth rates at 10 °C. The reason for the large deviation between actual and predicted specific growth rates may result from relatively large variations in the concentration of undissociated lactic acid in this type of mozzarella, with reported pH values varying between 4.9 and 6.2 for this type of cheese (Supplementary Table A.1). The high actual specific growth rates were extracted from Tirloni et al. (2019) who reported very low

Table 3
Overview of listeriosis outbreaks in 1983–2019 that were related to consumption of cheese. Compiled from EFSA reports (Eurosurveillance), CDC reports, Google and a general search in Web of Science and Scopus (keywords 'listeriosis' and 'cheese', or 'Listeria', 'outbreak' and 'cheese', sorted on relevance, first 300 hits).

Year of outbreak	Cheese group	Cases	Case-fatality rate (%)	Reference
1983–1987	Soft cheese made from raw milk	122	27	Bula et al. (1995)
1985	Mexican-style soft cheeses	142	34	Linnan et al. (1988)
1989	Camembert	2	0	Ries et al. (1990)
1989/1990	Blue cheese	26	23	Jensen et al. (1994)
1995	Brie de Meaux	NR	NR	Goulet et al. (1995)
1995	Soft cheese	37	30	Vaillant et al. (1998)
1997	Soft cheese	14	0	Jacquet et al. (1999)
1997	Soft cheese	NR	NR	RNSP (1997)
2000	Mexican-style cheese	13	0	Cartwright et al. (2013)
2001	Soft cheese	33	0	Carrique-Mas et al. (2003)
2001	Soft cheese	120	NR	Danielsson-Tham et al. (2004)
2001	Cheese	19	0	Makino et al. (2005)
2002	Cheese made from pasteurized milk	86	0	Pagotto et al. (2006)
2003	Soft cheese from raw milk	17	0	Gaulin et al. (2003)
2005	Mexican-style cheese	23	22	MacDonald et al. (2005)
2005	Queso fresco	9	NR	FIOD (2005)
2005	Tomme cheese	10	50	Bille et al. (2006)
2006	Soft cheese	189	14	Koch et al. (2010)
2006	Soft cheese	78	17	EFSA (2007)
2007	Camembert cheese	17	18	Johnsen et al. (2010)
2007	Mature cheese	NR	NR	Vít et al. (2007)
2008	Cheese	92	NR	Taillefer et al. (2010)
2008	Brie cheese	90	4	NBC (2008)
2008	Mexican-style cheese	8	0	Cartwright et al. (2013)
2009–2012	Queso fresco	30	37	Magalhaes et al. (2015)
2009–2011	Smear-ripened cheese (Tallegio)	NR	NR	Amato et al. (2017)
2010	Soft cheese (Panela, Queso fresco, Requeson)	5	0	FIOD (2010)
2010	Soft cheese	28	11	FIOD (2015)
2010	Acid-curd cheese (Quargel)	14	36	Fretz et al. (2010)
2011	Fresh cheese (chives)	2	NR	FIOD (2011)
2011	Mexican-style cheese	7	29	Jackson et al. (2011)
2011	Pave de Nord (Mimolette-type cheese made from pasteurized milk)	12	33	Yde et al. (2012)
2012	Latin-style fresh cheese (pasteurized milk)	2	0	De Castro et al. (2012)
2012	Ricotta cheese	22	18	CDC (2012)
2013	Camembert / Brie	18	17	Newsdesk (2013)
2014	Fresh cheese	1	13	CDC (2014a)
2014	Soft cheese	5	20	CDC (2014b)
2015	Cheese made from unpasteurized milk	2	0	Del-Valdivia-Tapia et al. (2015)
2015	Fresh cheese	3	33	FIOD (2015)
2017	Smear cheese	8	25	CDC (2017)
2019	Deli sliced cheese (suspected)	10	10	CDC (2019)

NR: Not reported.

concentrations of undissociated lactic acid in mozzarella (0–2.37 mM; when predictions are based on such lactic acid concentrations, no deviation between actual and predicted rates existed).

We reviewed undissociated lactic acid as a key parameter for growth inhibition of *L. monocytogenes* in many types of cheese. Therefore, it seems justified that RTE cheeses are classified based on their undissociated lactic acid concentration, and not only on pH and a_w. For cheeses such as Emmental, other inhibitory factors need further substantiation. The importance of undissociated lactic acid is underlined by the calculated Gamma factors for 10 cheese types using a MIC of undissociated lactic acid for *L. monocytogenes* of 6.35 mM and average values for total lactic acid content, moisture content, fat content, pH, a_w and temperature (Table 2). This calculation showed that in three out of 10 cheese types (namely, feta, Cheddar and Gouda), the Gamma factor for undissociated lactic acid is 0, implying that undissociated lactic acid alone fully inhibits growth of *L. monocytogenes* in these three cheese types.

In Table 4, a suggested categorization according to EU regulation EC 2073/2005 for 10 RTE cheese types is presented. The categorization is based on whether or not the consumption of the cheese was associated with identified cases of listeriosis in the past, and whether or not growth rates extracted from challenge studies and those predicted based on growth-inhibiting factors were positive or not. Ricotta, queso fresco, Camembert, high-moisture mozzarella, cottage and blue cheese were all linked to listeriosis (Table 3) and at the same time able to support growth of *L. monocytogenes* (Fig. 2). The data presented lend support to categorization of Gouda, feta and Cheddar as a category 1.3 food with levels not exceeding 100 colony forming units per gram (cfu/g) in five samples ($n = 5$) for products placed on the market during their shelf-life. Feta has an average pH of 4.65 and a total lactic acid content of 14 g per kg cheese; the undissociated lactic acid concentration is thus calculated to be 29.8 mM (Table 2). Cheddar has an average pH of 5.2 and a total lactic acid content of 15.0 g per kg cheese, resulting in an undissociated lactic acid concentration of 13.9 mM (Table 2). In all challenge studies with Cheddar and feta, no growth of *L. monocytogenes* was observed (Fig. 2), except for the study by Cui et al. (2016). The concentrations of undissociated lactic acid in the cheeses in their study are unknown, as acid content, pH, and salt content were not specified and there may have been mould growth (resulting in pH increase) as a commercial product was used. For high-moisture mozzarella, growth was observed and predicted, therefore categorization as a 1.2 food is suggested, especially in high-moisture mozzarella-type cheeses with pH >5.18 (then <6.35 mM undissociated lactic acid is calculated). For category 1.2 foods, the following food safety criteria apply: The cheeses queso fresco and Camembert can be categorized as category 1.2 foods, requiring absence of *L. monocytogenes* in 25 g (n = 5) before the food has left the immediate control of the food business operator, who has produced it. In queso fresco and Camembert, average pH values are 6.36 and 6.06 and average total lactic acid contents are 1.0 and 10.0 g per kg cheese, respectively, corresponding with undissociated lactic acid concentrations of 0.09 mM and 1.01 mM, respectively. As the calculated concentrations of undissociated lactic acid are much lower than the average and maximum MICs of 5.11 and 6.35 mM, respectively, for *L. monocytogenes* (Wemmenhove et al., 2016a), it is unlikely that undissociated lactic acid is an

Table 4

Classification of different types of cheese, with regards to food safety criteria. Classification is based on the association with listeriosis in the past (+ when linked), the minimum and maximum value for $\frac{\Delta log\text{N}\bullet ln(10)}{\text{t}}$ as calculated from data from challenge studies (+ when positive values were obtained; +/− when sometimes positive values and – when no positive values were obtained for $\frac{\Delta log\text{N}\bullet ln(10)}{\text{t}}$) and the predicted specific growth rates (μ) using the Gamma model with average values of the individual factors undissociated lactic acid, temperature (T), pH and a_w in the cheese as listed in Table 2, without including a lag time and $\mu_{opt} = 1.69\ h^{-1}$ and $0.73\ h^{-1}$ (equaling the maximum and average optimum growth rates for *L. monocytogenes* in milk as extracted from Combase (www.combase.cc) when searching for growth rates of *L. monocytogenes* in milk at 30–37 °C, and a MIC of undissociated lactic acid for *L. monocytogenes* of 6.35 mM according to Aryani et al. (2015).

Type of cheese	Cheese category and risk ranking according to FDA (2003)	Associated with identified cases of listeriosis in the past (based on Table 3)?	Minimum and maximum $\frac{\Delta logN\bullet ln(10)}{t}$ (h^{-1}) extracted from challenge studies. If positive, this results in a '+'.	μ (h^{-1}) calculated with the Gamma model taking the average values for undissociated lactic acid, temperature, pH and a_w, each growth inhibitor from Table 2, and $\mu_{opt} = 0.73\ h^{-1}$ or $\mu_{opt} = 1.69\ h^{-1}$. If >0, this results in a '+'.	Suggestion for category (based on association with listeriosis, maximum $\frac{\Delta logN\bullet ln(10)}{t}$ and μ values.
Ricotta	Soft unripened - > moderate risk	Yes	0.0049 to 0.32 +	0.067 or 0.16 +	1.2, absence in 25 g (n = 5) before the food has left the immediate control of the food business operator, who has produced it, and not exceeding 100 cfu/g for the products placed on the market during its shelf-life.
Queso fresco	Fresh soft - > low risk	Yes	−0.0089 to 0.25 +/−	0.049 or 0.11 +	1.2, absence in 25 g (*n* = 5) before the food has left the immediate control of the food business operator, who has produced it, and not exceeding 100 cfu/g for the products placed on the market during its shelf-life.
Camembert	Soft ripened - > low risk	Yes	−0.0062 to 0.120 +/−	0.018 or 0.042 +	1.2, absence in 25 g (*n* = 5) before the food has left the immediate control of the food business operator, who has produced it, and not exceeding 100 cfu/g for the products placed on the market during its shelf-life.
Cottage	Soft unripened - > low risk	No	−0.13 to 0.040 +/−	0.044 or 0.102 +	1.2, absence in 25 g (*n* = 5) before the food has left the immediate control of the food business operator, who has produced it, and not exceeding 100 cfu/g for the products placed on the market during its shelf-life.
Blue	Semi-soft - > low risk	Yes	−0.011 to 0.042 +/−	0.0031 or 0.0073 +	1.2, absence in 25 g (n = 5) before the food has left the immediate control of the food business operator, who has produced it, and not exceeding 100 cfu/g for the products placed on the market during its shelf-life.
High-moisture mozzarella	Soft ripened - > low risk	No	0.017 to 0.312 +	0.0013 or 0.0030 +	1.2, absence in 25 g (n = 5) before the food has left the immediate control of the food business operator, who has produced it, and not exceeding 100 cfu/g for the products placed on the market during its shelf-life.
Emmental	Hard - > low risk	No	−0.029 to −0.00085 -	0.0031 or 0.073 +	1.2, absence in 25 g (n = 5) before the food has left the immediate control of the food business operator, who has produced it, and not exceeding 100 cfu/g for the products placed on the market during its shelf-life. But possibly 1.3 with not exceeding 100 cfu/g (n = 5) for products placed on the market during their shelf-life, if there is enough scientific proof that factors that are present in this type of cheese but that are not included in the predictions presented in this article (e.g. propionic acid; acetic acid; free fatty acids), can inhibit growth of *L. monocytogenes* in this type of cheese.
Gouda	Semi-soft - > low risk	No	−0.0033 to −0.0002 -	0 or 0 -	1.3, not exceeding 100 cfu/g (n = 5) for products placed on the market during their shelf-life
Cheddar	Hard - > low risk	No	−0.067 to 0.062 -	0 or 0 +/−*	1.3, not exceeding 100 cfu/g (n = 5) for products placed on the market during their shelf-life
Feta	Soft ripened - > low risk	No	−0.049 to −0.00055 -	0 or 0 -	1.3, not exceeding 100 cfu/g (n = 5) for products placed on the market during their shelf-life

* +/−, because 2 out of 45 specific growth rates obtained from literature were positive. On the surface of commercial Cheddar, 2 positive growth rates were obtained by Cui et al. (2016), but the validity of these data is questionable, as no data on pH, a_w or possible mould growth were available.

important growth-inhibiting factor in these cheeses. In practice, queso fresco and Camembert cheeses have been linked with listeriosis cases on several occasions (Table 3), and growth of *L. monocytogenes* was supported in challenge studies that were performed with these cheeses.

When a food is categorized as an RTE food product able to support growth of *L. monocytogenes*, stringent control measures, supplemented with sampling for verification, need to be in place. In such cheeses, heat treatment of the raw milk becomes an even more important factor to reduce the risk of contamination with *L. monocytogenes*. Pasteurization is an effective measure to reduce the risk of contamination of cheese products with *L. monocytogenes* via raw milk. A minimal heat treatment of 15 s at 72 °C leads to an average reduction of 10.4 log cfu/g based on an average D value and by 2.7 log cfu/g based on a 95% upper confidence interval for the D value (den Besten and Zwietering, 2012).

In addition, food safety risks related to cheeses that can support growth of *L. monocytogenes* can be decreased by applying additional hurdles and different storage regimes (e.g. shorter shelf life, refrigerated storage, protective cultures) (Coelho et al., 2014; Maisnier-Patin et al., 1992; Davies et al., 1997; Soni et al., 2010; Soni et al., 2012; Spanu et al., 2012). In the case of cottage cheese, sorbic acid may be added which inhibits growth of *L. monocytogenes* (Østergaard et al., 2014; Østergaard et al., 2015). Those additional hurdles and storage regimes are very cheese-specific and not included in our model.

Undissociated lactic acid has been shown to play a major role in the inhibition of growth of *L. monocytogenes* in various cheeses. In the cheeses Cheddar, feta and Gouda, undissociated lactic acid is expected to lead to full growth inhibition. A low water activity, such as in blue cheese, can be an additional critical factor for growth, and can inhibit growth of *L. monocytogenes* together with temperature and pH. In cheeses associated previously to listeriosis such as Camembert, ricotta and cottage cheese, there is insufficient undissociated lactic acid for full growth inhibition, and therefore additional factors for growth inhibition are required.

To safeguard the safety of cheeses with respect to *L. monocytogenes*, critical control points such as pasteurization must be validated and verified, post-processing contamination prevented, and product composition and storage conditions designed so that levels do not exceed specified limits. The presented model will be a valuable tool to assess potential outgrowth of the pathogen in cheeses that contain lactic acid as the main acid.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2021.109350.

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