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Short communication

Minimal inhibitory concentrations of undissociated lactic, acetic, citric and propionic acid for *Listeria monocytogenes* under conditions relevant to cheese

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ABSTRACT

Minimal inhibitory concentrations (MICs) of undissociated lactic acid were determined for six different *Listeria monocytogenes* strains at 30 °C and in a pH range of 4.2–5.8. Small increments in pH and acid concentrations were used to accurately establish the growth/no growth limits of *L. monocytogenes* for these acids. The MICs of undissociated lactic acid in the pH range of 5.2–5.8 were generally higher than at pH 4.6 for the different *L. monocytogenes* strains. The average MIC of undissociated lactic acid was 5.0 (SD 1.5) mM in the pH range 5.2–5.6, which is relevant to Gouda cheese. Significant differences in MICs of undissociated lactic acid were found between strains of *L. monocytogenes* at a given pH, with a maximum observed level of 9.0 mM. Variations in MICs were mostly due to strain variation. In the pH range 5.2–5.6, the MICs of undissociated lactic acid were not significantly different at 12 °C and 30 °C. The average MICs of undissociated acetic acid, citric acid, and propionic acid were 19.0 (SD 6.5) mM, 3.8 (SD 0.9) mM, and 11.0 (SD 6.3) mM, respectively, for the six *L. monocytogenes* strains tested in the pH range 5.2–5.6. Variations in MICs of these organic acids for *L. monocytogenes* were also mostly due to strain variation. The generated data contribute to improved predictions of growth/no growth of *L. monocytogenes* in cheese and other foods containing these organic acids.

1. Introduction

Listeria monocytogenes is a Gram-positive foodborne pathogen (Vazquez-Boland et al., 2001). Growth of this bacterium can be inhibited effectively with short-chain organic acids in their undissociated form; these can pass through the bacterial cell membrane. The acid subsequently dissociates in the cytoplasm, resulting in an increased hydrogen ion concentration in the cell. To restore the intracellular pH, the hydrogen ions are pumped out, but this disturbance of the proton motive force is an energetically unfavorable process for bacteria (Mitchell, 1961).

The concentration of undissociated acid in watery solutions is determined by the total concentration of the acid, the pH, and the dissociation constant pK_a. Minimal inhibitory concentrations (MICs) of the undissociated forms of lactic acid, acetic acid, citric acid and propionic acid for *L. monocytogenes* have been reported in various studies (Ahamad and Marth, 1989; Aryani et al., 2015; Chen and Shelef, 1992; Conner et al., 1990; Coroller et al., 2005; Houtsma et al., 1993; Van der Veen et al., 2008; Vasseur et al., 1999; Young and Foegeding, 1993). However, the data on MICs of undissociated acids for *L. monocytogenes* available in literature are limited, varying from 2 to 46 data points per acid. In a number of studies available in literature, relatively large intervals between undissociated acid concentrations were used, resulting in quite rough estimations of the actual lowest concentrations that inhibit growth (Ahamad and Marth, 1989; Conner et al., 1990; Coroller et al., 2005; Van der Veen et al., 2008). Furthermore, a large variability in MIC values between different strains of *L. monocytogenes* has been observed in previous studies (Coroller et al., 2005; Van der Veen et al., 2008).

To allow for prediction of growth/no growth of *L. monocytogenes* in foods that contain short-chain organic acids, more

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comprehensive datasets with MICs of these acids (using small increments in total acid concentrations and pH values) are needed for a variety of *L. monocytogenes* strains. For instance, in the case of Dutch-type cheeses like Gouda, Edam and Maasdam, made from pasteurized milk with rennet-induced curd formation, it is known that a low pH and the presence of short-chain organic acids are important factors contributing to inhibition of growth of *L. monocytogenes* (Millet et al., 2006).

The aim of this study was to establish accurate minimum inhibitory concentrations of undissociated lactic acid, acetic acid, citric acid and propionic acid for six different strains of *L. monocytogenes*. The inhibitory effects of the undissociated acids were established in a range of pH values from 4.2 to 5.8, thus including conditions that are relevant to Dutch-type cheeses. In addition, the effects of different salts that are present in cheese were tested in the presence of lactate. MICs of the different acids for *L. monocytogenes* were determined at 30 °C, reflecting normal processing temperatures for cheese-making, and in addition, MICs of lactate were established at 12 °C, reflecting cheese ripening temperatures.

2. Materials and methods

2.1. Listeria monocytogenes strain selection and cultivation

Six strains of *L. monocytogenes* were selected from the NIZO culture collection (containing 138 *L. monocytogenes* strains) based on the origin of the strains, the serotype and the resistance to lactic acid, which were previously established (Van der Veen et al., 2008). The following strains were selected, based on reported MICs of undissociated lactic acid in Van der Veen et al. (2008): *L. monocytogenes* strain 1F (1/2a, cheese isolate, reported MIC 7.5 mM), 2F (1/2a, cheese isolate, reported MIC 4.0 mM), 6E (1/2a, isolate from wall in cheese factory, reported MIC 7.5 mM), EGDe (1/2a, rabbit isolate, reported MIC 2.5 mM), L4 (1/2b, milk isolate, reported MIC 7.5 mM) and Scott A (4b, outbreak isolate linked with milk, reported MIC 2.5 mM); see also Tables S1 and S3 in Van der Veen et al. (2008). The strains were cultured individually in Brain Heart Infusion (BHI, Merck, Darmstadt, Germany) for ~19 h and the initial OD of the individual strains was normalized by varying the culturing time at 30 °C before exposure to acid.

2.2. Generation of datasets to determine MICs of organic acids for L. monocytogenes

Four datasets were generated to determine the MICs of organic acids in BHI (Supplementary Table S1).

Dataset 1 was generated to establish the MICs of undissociated lactic acid for six different *L. monocytogenes* strains (1F, 2F, 6E, EGDe, L4, Scott A), using final total concentrations of 0.009, 0.013, 0.020, 0.030, 0.040, 0.046, 0.059, 0.071, 0.088, 0.11, 0.13, 0.18, 0.21, 0.32, 0.50, or 0.79 M, at nine different pH values (ranging from 4.2 to 5.8 with intervals of 0.2), and one temperature in two independent experiments with three replicates per experiment. Incubation was performed at 30 °C up to one month, reflecting normal processing temperatures during cheese-making.

As Dutch-type cheese contains sodium chloride and calcium chloride, dataset 2 was generated to determine the effect of addition of salt on the MIC of lactate for *L. monocytogenes*. The MIC of lactate was determined for the same concentration interval of lactate and for the same six *L. monocytogenes* strains as in dataset 1, but using pH values ranging from 5.2 to 5.6 with intervals of 0.2, and in the absence or in the presence of salts (0.20 M sodium chloride, 0.36 M calcium chloride and 0.20 M sodium chloride + 0.36 M calcium chloride in dataset 2).

Dataset 3 was generated to establish the MICs of undissociated acetic acid, citric acid and propionic acid for *L. monocytogenes*. The same six *L. monocytogenes* strains were used as in dataset 1, using pH values ranging from 5.2 to 5.6 with intervals of 0.2, and the following final total acid concentrations: acetic acid, 0.006, 0.014, 0.029, 0.032, 0.043, 0.057, 0.071, 0.081, 0.086, 0.16, 0.24, 0.32, 0.40, or 0.49 M; citric acid, 0.014, 0.14, 0.35, 0.71, 2.13 M; propionic acid 0.0077, 0.015, 0.031, 0.062, 0.093, 0.12, 0.15, 0.19 M.

Dataset 4 was generated to investigate whether lowering the temperature to that of cheese ripening would influence the MIC of organic acids. In dataset 4, the MICs of lactate were determined as in dataset 1, but generated in one single experiment with three replicates and incubated at 12 °C (cheese ripening temperature) up to three months. The MICs of lactate of dataset 4 were generated simultaneously with the first duplicate determination of the MICs of lactate of dataset 1, incubated at 30 °C up to one month. The simultaneous assessment at 12 °C and 30 °C allowed for a comparison of the MICs at those temperatures.

2.3. Determination of the MICs of undissociated organic acids for L. monocytogenes

Culture medium with different pH values and concentration ranges of organic acids were prepared as follows. Equimolar stock solutions of an organic acid and of its potassium salt (both dissolved in water) were made at 2.1 times the final target concentrations (as listed above in paragraph 2.2) and mixed to obtain the desired pH (*i.e.* 4.2 to 5.8 with intervals of 0.2 for datasets 1 and 4, and 5.2 to 5.6 with intervals of 0.2 for dataset 2 and 3). The 2.1-fold concentrated organic acid solutions (with different organic acid concentrations and pH values) were filter-sterilized using Nalgene 0.2 μm filters (Sigma Aldrich, Seele, Germany) and 95 μL aliquots were added to wells of 96-well plates containing 95 μL of two-fold concentrated filter sterilized BHI that was adjusted with hydrochloric acid (Sigma Aldrich) to the same pH as the organic acid solution that was added. The final pH was recorded prior to incubation. Finally, 10 μL of a bacterial inoculum in BHI was added. The bacterial inoculum was obtained by culturing six strains of *L. monocytogenes* individually overnight at 30 °C to an OD_{630} of approximately 1.0 (~$3.6 \cdot 10^9$ cfu mL^{-1}). Cells from 100 μL of the culture were concentrated by centrifugation (15 min, 5000× g), and the cell pellet was resuspended in 10 mL BHI of which the pH was adjusted to match the pH used in the experiments to determine the MICs. Thevolume of 10 μL of the bacterial inoculum was suspended in the 96-well plates (in wells with corresponding pH values) to obtain final concentrations of individual *L. monocytogenes* strains of ~$1.8 \cdot 10^6$ cfu mL^{-1} as determined by plate counts on BHI agar (Sigma Aldrich, Seele, Germany) in 200 μL final volumes. Incubation was performed at 30 °C and growth of *L. monocytogenes* was monitored in triplicate in two independent experiments by measurements of the optical density at 630 nm (OD_{630}) using an EL808 IU-PC spectrophotometer (Bio-Tek, Winooski, Vermont, USA) throughout a 1-month incubation period. Growth was defined as an increase in OD_{630} greater than 0.05 compared with the negative control. To confirm the growth/no growth limits that were determined based on optical density readings, counts in the wells with an OD_{630} increase less than 0.05 were determined by plating on BHI agar and compared with the initial inoculum level.

MICs of undissociated organic acid for *L. monocytogenes* were calculated based on equation (1), which was derived from the Henderson–Hasselbalch equation.

$$[Undissociated\ acid] = \frac{[Total\ acid]}{1 + 10^{pH - pK_a}} \quad (1)$$

The molarities of the total acid solutions were pre-set, and the pH values used in these calculations were determined prior to incubation. The equilibrium constants K for ionization of the undissociated form of the different acids were established previously (Dawson et al., 1986), *i.e.* pK_a 3.86 for lactic acid, 4.76 for acetic acid, 3.13 for citric acid and 4.86 for propionic acid.

2.4. Literature search

Previously reported MICs of undissociated lactic acid, acetic acid, citric acid and propionic acid were obtained from literature (first 200 hits in Scopus, sorted on relevance). The following keywords were used in the search: (*L. monocytogenes* AND MIC AND [undissociated acid OR organic acid OR lactic acid OR acetic acid OR citric acid OR propionic acid OR lactate OR acetate OR citrate OR propionate]). Furthermore, a literature search was performed using Web of Science and Google Scholar.

2.5. Statistical analysis

A general linear model ANOVA (between subjects design, pairwise comparison, Product Tukey test at 95%) was constructed in Minitab (Minitab Inc., Pine Hall, Pennsylvania, USA) to determine if the MIC was determined by the fixed factors of study or by random variation. Datasets 1, 2 and 3 consisted of data from two independent experiments. For those datasets, 'experiment' and 'replicates' were defined as random factors. Fixed factors were 'pH' and 'strain' in dataset 1, 'pH', 'strain' and 'type of salt' in dataset 2, and 'pH', 'strain' and 'type of organic acid' in dataset 3. In dataset 4, the MICs of undissociated lactic acid were determined at pH values ranging from 4.2 to 5.8 at 12 °C and compared with the MICs at 30 °C of the first duplicate determination of dataset 1. 'Replicates' were treated as random factor and 'pH', 'strain' and 'temperature' were treated as fixed factors in the ANOVA analysis of dataset 4. Residual plots and boxplots were constructed to check for normality and outliers. Post-hoc ANOVA tests (Product Tukey test at 95%) were performed using Fizz software (Biosystèmes, Couternon, France) to determine the factors significantly affecting the MIC.

3. Results & discussion

In this study, the MIC values of undissociated lactic acid, acetic acid, citric acid and propionic acid were established for the six *L. monocytogenes* strains at pH 4.2–5.8. No growth was observed at pH values 4.2 or 4.4 for any of the strains, independent of the absence or presence of organic acids (Fig. 1). This is in line with the lowest pH growth limit reported by ICMSF (1996), which is pH 4.4 for *L. monocytogenes*.

The MICs of undissociated lactic acid for *L. monocytogenes* showed a pH dependency, with significantly lower values at pH 4.6 than at pH 5.2, 5.6 and 5.8 for strains 1F, EGDe, L4 and Scott A and significantly higher values at pH 5.8 than at pH 4.6-5.6 for strains 2F and 6E (Fig. 1 and Supplementary Table S2). Between strains, significant differences in MICs of undissociated organic acid at a given pH were seen in all four datasets generated (Supplementary Table S1). More specifically, the MICs of undissociated lactic acid showed significant differences between strains of *L. monocytogenes* at pH 4.8, 5.0, 5.2, 5.4 and 5.6, but not at pH 4.6 and pH 5.8 (Supplementary Table S3). Relatively high standard deviations were observed for MICs at low pH values (Fig. 1, Supplementary Tables S2 and S3). This likely results from a heterogeneous response to severe acid stress of cells within a population, with only certain cells able to overcome this stress and grow (this phenomenon was recently reviewed by Abee et al., 2016). In addition, some high standard deviations were found for MICs at pH 5.6 in the presence of high

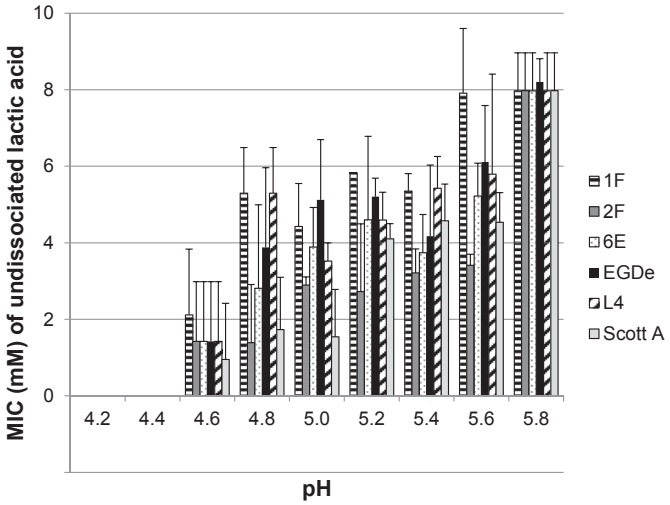

Fig. 1. Average MIC of undissociated lactic acid (mM) for *L. monocytogenes* strains 1F, 2F, 6E, EGDe, L4 and Scott A at pH values ranging from 4.2 to 5.8, determined in BHI at 30 °C. Growth of *L. monocytogenes* was only observed at pH values higher than 4.4. At pH 4.2 and 4.4 growth was not observed in the absence or presence of lactic acid. Upper error bars are used to show the standard deviation of six measurements (n = 6 for each strain and for each pH).

concentrations of undissociated acids; here, the variation is likely due to increasing intervals between the concentrations at the top end of the range.

At pH values relevant to Gouda cheese, namely pH 5.2, 5.4 and 5.6, no significant differences were observed between the MICs of undissociated lactic acid per individual strain at these three pH values, except in the case of strain 1F (Supplementary Table S2). For the individual strains, the MIC data generated at pH 5.2, 5.4 and 5.6 were subsequently combined in further analyses.

Differences between strains were observed: MIC values of undissociated lactic acid were significantly higher for *L. monocytogenes* strain 1F (average MIC of 6.1 mM) than for strains 2F, 6E and Scott A (average MICs of 3.0, 5.1 and 4.3 mM, respectively), but not significantly higher or lower than the MICs for strains EGDe and L4 (average MICs of 5.5 and 5.6 mM) (Supplementary Table S4). These findings indicate that strain variation contributes significantly to the observed variation in the concentrations of lactic acid that are required to inhibit growth. The average MIC of undissociated lactic acid determined at pH 5.2–5.6 for all six strains combined was 5.0 mM with a standard deviation of 1.5 mM, as seen in dataset 1 and 2 (Supplementary Table S4). The highest value observed in this pH range was 9.0 mM (Fig. 1).

The cumulative frequency plot of the MICs of undissociated lactic acid at pH 5.2–5.6 obtained in this study and MICs reported in literature are presented in Fig. 2A. The previously reported MICs are in the same range as the ones determined in this study. The small increments in consecutive concentrations of undissociated lactic acid used in this study resulted in more accurate estimations of the actual MICs for *L. monocytogenes* (Fig. 2A).

At a temperature commonly used during cheese ripening, namely 12 °C, the MICs of undissociated lactic acid for *L. monocytogenes* after one month of incubation were not significantly different ($p > 0.05$) from those determined after incubation for one month at 30 °C in the pH range 5.2–5.6. When taking all data over the full pH range tested into account, *i.e.* at pH 4.6-5.8, the average MICs were slightly lower at 12 °C (3.2 mM on average) than at 30 °C (3.5 mM on average) (see Supplementary Table S1, $p = 0.02$). Similar effects were seen by Houtsma (1996) who reported lower MICs of undissociated lactic acid for *L. innocua* at 4 °C

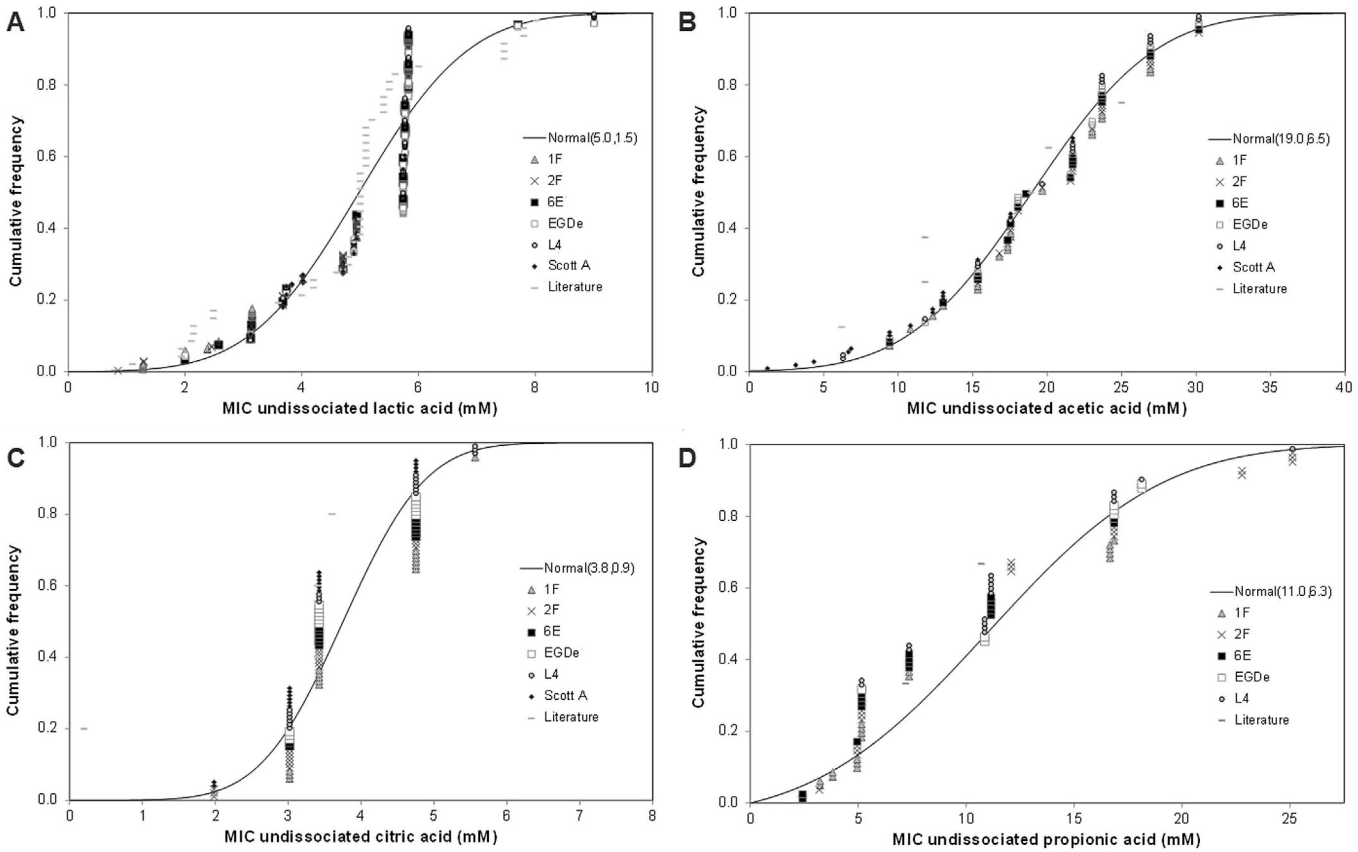

Fig. 2. Cumulative frequency plot of the MICs of undissociated lactic acid (A), acetic acid (B), citric acid (C) and propionic acid (D) for *L. monocytogenes* strains 1F, 2F, 6E, EGDe, L4 and Scott A determined at pH 5.2, 5.4 and 5.6 and plotted individually. Furthermore, literature values of the MICs of undissociated acids for *L. monocytogenes* strains have been included, as reported by Ahamad and Marth (1989), Aryani et al. (2015), Chen and Shelef (1992), Conner et al. (1990), Coroller et al. (2005), Houtsma et al. (1993), Van der Veen et al. (2008), Vasseur et al. (1999) and Young and Foegeding (1993). In the study by Van der Veen et al. (2008), MICs of undissociated lactic acid were obtained for 138 strains of *L. monocytogenes*, but only the MICs of strains 1F, 2F, 6E, EGDe, L4, Scott A were incorporated in this figure to avoid a skew in the literature dataset due to one study. The experimental data generated in this study have been fitted with Normal distributions (average with standard deviations) and were 5.0 (SD 1.5) mM for undissociated lactic acid, 19.0 (SD 6.5) mM for undissociated acetic acid, 3.8 (SD 0.9) mM for undissociated citric acid and 11.0 (SD 6.3) mM for undissociated propionic acid.

after three weeks than at 10, 20, or 30 °C.

MICs of undissociated lactic acid for strains EGDe and Scott A were significantly lower in the presence of sodium chloride together with calcium chloride at concentrations relevant to cheese (0.20 M and 0.36 M, respectively) than in the absence of these added salts or in the presence of these salts added individually. For the other four strains, no significant differences were observed (results not shown). Hence, the effect of sodium chloride and calcium chloride on the MIC of undissociated lactic acid for *L. monocytogenes* in cheese appears overall limited, but may be strain dependent.

For undissociated acetic acid, the average MIC for all six *L. monocytogenes* strains tested in pH range 5.2–5.6 was 19.0 mM with a standard deviation of 6.5 mM. The highest MIC observed was 30.2 mM (Fig. 2B). The average MIC at pH 5.6 was 20.7 mM, which was significantly higher than the average MICs of undissociated acetic acid at pH 5.2 and 5.4 (18.0 and 17.7 mM). The consecutive MIC intervals of acetic acid were larger at pH 5.6 than at pH 5.2 and 5.4 (data not shown), which might result in greater deviations between the determined and the true MICs at pH 5.6 than at pH 5.2 and 5.4. The MIC for *L. monocytogenes* strain Scott A (11.6 mM) was significantly lower than the MICs for strains 1F, 2F, 6E, EGDe and L4 (18.3–21.8 mM; see Supplementary Table S4). The MICs of undissociated acetic acid are in line with the MICs in previous studies for *L. monocytogenes* (Fig. 2B), which varied from 6.2 to 43.9 mM, with an average of 19.7 mM (Ahamad and Marth, 1989; Conner et al., 1990; Coroller et al., 2005; Vasseur et al., 1999; Young and Foegeding, 1993).

For undissociated citric acid, the average MIC value for all six strains of *L. monocytogenes* tested in pH range 5.2–5.6 was 3.8 mM with a standard deviation of 0.9 mM (Supplementary Table S4) and a highest MIC of 5.6 mM (Fig. 2C). The MIC for strain 2F (3.3 mM on average) was significantly lower than that of strains 1F and L4 (4.0 and 4.1 mM on average). For strains 6E, EGDe and Scott A, the MIC values of undissociated citric acid (3.4–3.9 mM) did not differ significantly from the MICs for 1F, 2F and L4 (Supplementary Table S4). The MICs determined for undissociated citric acid in this study are similar to or higher than the previously reported MICs of 3.4 mM (Ahamad and Marth, 1989), 0.2, and 3.6 mM (Coroller et al., 2005). The MIC reported by Ahamad and Marth (1989) was determined at pH 4.5, which is near the pH limit for growth of *L. monocytogenes*. This could explain the lower MIC observed in that study compared with those found in this study. Coroller et al. (2005) determined the MICs of undissociated citric acid at a concentration range of 0–300 mM total citric acid and at pH 5.0 to 7.0. Only the lowest and highest MICs of undissociated citric acid were reported, but not the consecutive concentrations of undissociated citric acid tested. This complicates a direct comparison between MIC values of undissociated citric acid obtained by Coroller et al. (2005) and the ones established in this study.

For undissociated propionic acid, the average MIC for the *L. monocytogenes* strains tested in pH range 5.2–5.6 was 11.0 mM

with a standard deviation of 6.3 mM (Supplementary Table S4). The highest value observed was 25.1 mM (Fig. 2D). The MICs for strains 1F and 6E (8.2 and 8.3 mM on average) were consistent with the MICs previously determined by Coroller et al. (2005) of 7.2 and 10.7 mM (Fig. 2D), but were significantly different from the MICs for strains 2F and EGDe (average MICs of 13.4 and 15.3 mM, respectively). The MIC of undissociated propionic acid for strain L4 (average MIC of 12.2 mM) was not significantly different from the other strains (Supplementary Table S4).

Growth of *L. monocytogenes* has not been observed in challenge studies using Gouda and Swiss-type cheese; in fact, inactivation was observed after extended ripening times (Bachmann and Spahr, 1995; Buazzi et al., 1992; Northolt et al., 1988; Wemmenhove et al., 2013, 2014). In Dutch-type cheeses, several short-chain organic acids can be found, such as lactic acid, acetic acid, citric acid and propionic acid. Cow's milk naturally contains 0.05–0.8 mM acetate, 9 mM citrate and <0.4 mM lactate (total acid) and has a pH of around 6.5 to 6.7 (Walstra and Jenness, 1984). It also contains lactose, which starter lactic acid bacteria used in cheese manufacturing can convert to lactic acid (Fox et al., 2004a). In Gouda cheese ripened for 4 weeks or longer, concentrations of total lactic acid and acetic acid were 13.9 and 1.1 g kg^{-1}, respectively, whereas citric acid and propionic acid were absent (Wemmenhove et al., 2013). The estimated concentration of undissociated lactic acid in the water phase of Gouda cheese is 9.2 mM (Supplementary Table S5), thus higher than the average and highest MICs needed for full inhibition of growth of *L. monocytogenes* (*i.e.* 5.0 mM and 9.0 mM, respectively). The estimated concentration of undissociated acetic acid in Gouda is 9.0 mM (Supplementary Table S5), which is lower than the average MIC (19.0 mM) needed for full inhibition of growth of *L. monocytogenes*. Therefore, lactic acid appears to be a critical factor for inhibition of growth of this pathogen in Gouda cheese.

In semi-hard cheeses with propionic acid fermentation, such as Maasdam and Swiss-type cheese, propionic acid bacteria convert the lactic acid produced by lactic acid bacteria to other organic acids during ripening, resulting in an increase of the pH from 5.3 to 5.8 (Fox et al., 2004b). Total concentrations of 3.5 g lactic acid per kg cheese, 2.0 g acetic acid per kg cheese and 5.0 g propionic acetic acid per kg cheese have been reported in a 35-day old Swiss-type cheese (Fox et al., 2004a). This results in estimated concentrations of 0.95 mM undissociated lactic acid, 8.1 mM undissociated acetic acid, and 17.3 mM undissociated propionic acid in the water phase of the cheese, respectively (Supplementary Table S5). Given the MICs of these acids (average 5.0 mM, 19.0 mM and 11.0 mM undissociated acid, respectively), propionic acid is thought to play an important role in inhibition of growth of *L. monocytogenes* during ripening of such cheeses.

4. Conclusions

MICs of undissociated lactic acid for six strains of *L. monocytogenes* were generally lower at pH values approaching the pH limit for growth of this bacterium (*i.e.* pH 4.4) than at pH values above 5.2. MICs of undissociated lactic acid, acetic acid, citric acid and propionic acid for *L. monocytogenes* at pH values relevant to cheese (in the range 5.2–5.6) showed variations for each of the acids, which could mostly be attributed to strain variation. Undissociated lactic acid is expected to have a substantial inhibitory effect on growth of *L. monocytogenes* in Gouda cheese, whereas propionic acid is expected to play an important role in semi-hard cheeses that undergo propionic acid fermentation. The generated data on MICs of undissociated organic acids in Dutch-type cheeses for different strains of *L. monocytogenes* can be used to improve risk assessments.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fm.2016.03.012.

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