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Short communication: *In situ* evaluation of citric acid powder to control *Listeria monocytogenes* on floors in meat processing plants

Kurzmitteilung:
In situ Untersuchung der Verwendung von Zitronensäurepulver zur Kontrolle von Listeria monocytogenes auf Fussböden in der Fleischwarenindustrie

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Summary

Several guidelines for control of *L. monocytogenes* in food processing environments recommend the addition of citric acid powder to floors. However, documentation of the effect of this advice on *L. monocytogenes* in the food industry is not available in scientific literature. In the current study, the effect of addition of citric acid powder to five floors and a floor gutter, where water was observed to accumulate, on the occurrence of *L. monocytogenes* and total bacterial numbers were tested *in situ* in two meat processing plants. Overall, starting with addition of citric acid lead to a reduction of *L. monocytogenes* positive floors from 59 % to 13 %. *Listeria monocytogenes* was eradicated from all floor areas, with the exception of a floor that was positive in three out of four samplings in the citric acid test period. The total bacterial counts were on average lower in the period with addition of citric acid than in the control period. In the current study it was shown that addition of citric acid powder to floors where water tend to accumulate, can be effective for control of *L. monocytogenes*.

Keywords: *Listeria monocytogenes,* citric acid, disinfection, floor, cross-contamination, hygiene

Zusammenfassung

Mehrere aktuelle Richtlinien empfehlen die regelmäßige Verwendung von Zitronensäure in Pulverform zur Kontrolle von *Listeria monocytogenes* auf Fußböden in der Industrie. Allerdings gibt es in der wissenschaftlichen Literatur bis heute keine Veröffentlichung zur Wirksamkeit dieser Methode. In der hier präsentierten Arbeit wurde die Effektivität dieser Methode *in situ* in zwei Fleischwarenfabriken untersucht. Insgesamt sechs Testpunkte wurden ausgewählt – fünf Stellen auf Fußböden, wo sich während der Produktion und nach der Reinigung Wasser ansammelte, sowie ein Abfluss. Dort wurden das Auftreten von *L. monocytogenes* sowie die Gesamtkeimzahl bestimmt. Die Ergebnisse zeigten einen Rückgang der positiven Listerienproben von 59 % vor der regelmäßigen Verwendung von Zitronensäurepulver auf 13 % danach. An fünf der sechs Teststellen konnten nach der regelmäßigen Verwendung von Zitronensäure keine Listerien mehr nachgewiesen werden. Lediglich einer der sechs Punkte wies nach der Verwendung von Zitronensäure noch Listerien auf (drei von vier Proben positiv). Die Gesamtkeimzahl war in der Testperiode im Durchschnitt niedriger als in der Kontrollperiode vor der Verwendung von Zitronensäure. Zusammengefasst zeigt diese Studie, dass die regelmäßige Verwendung von Zitronensäure zur aktiven Kontrolle von *L. monocytogenes* an Problemstellen in der Industrie, wo sich Wasser ansammelt, verwendet werden kann.

Schlüsselwörter: *Listeria monocytogenes,* Zitronensäure, Desinfektion, Fußböden, Kreuzkontamination, Hygiene

Introduction

Listeria monocytogenes is a food borne bacteria that causes the disease listeriosis. The disease is especially linked to consumption of ready-to-eat (RTE) foods, including RTE meat products like deli meat, ham, sliced chicken etc., which become contaminated during production due to cross-contamination from surfaces/machines (Swaminathan and Gerner-Smidt, 2007). *L. monocytogenes* establish itself in processing environments and persistence of specific strains for years has been shown (Ferreira et al., 2014). Positive samples with *L. monocytogenes* are often associated with humid niches where *L. monocytogenes* may grow and survive. Floors and drains are among the areas with the highest prevalence of *L. monocytogenes* (Berrang et al., 2013; Ruckerl, et al., 2014). Although floors and drains are not in direct contact with food, they may act as sources of *L. monocytogenes* and can contaminate food or food contact surfaces e. g. from splashes during cleaning procedures, via equipment, hands or products that have been in contact with floors. Control of *L. monocytogenes* is challenging and it is often extremely difficult to eradicate *L. monocytogenes,* once established. Many guidelines and advices are available on how to control the bacterium in processing environments. In general, several of the advices are based upon practical experience and scientific evaluations may be scarce. Several guidelines recommend the use of citric acid powder on floors for controlling *L. monocytogenes* (Irish sea fisheries board, 2011; FAO, 1999; Tompkin et al., 1999). The guidelines cite no reference to data supporting this recommendation and to our knowledge there does not exist reported data on the efficiency of using citric acid powder to control *L. monocytogenes* on surfaces in the food industry. Also procedural details on how to use the citric acid powder are limited in the guidelines. Tompkin et al. (1999) and FAO (1999) recommend that powdered citric acid may be added to floors to control *L. monocytogenes,* and that it should be checked that the resulting pH is lower than 5.0, but no further details are provided. Citric acid is a three carboxylic acid with its lowest pK_a = 3.1. It is well known that citric acid has an antimicrobial effect against *L. monocytogenes* (Young & Foegeding, 1993), and can reduce its numbers in foods, including meat (Bal'a and Marshall, 1998; Gonzalez-Fandos et al., 2009; Over et al., 2009) and in cheese brines (Parikh et al., 2011).

In the present study, the effect of adding citric acid powder to five floors and a floor gutter on the occurrence of *L. monocytogenes* was tested in situ in two meat processing plants.

Material and methods

Two meat processing plants were included in the study. Two floor sites and a floor gutter from plant A (samples A1–A3), and three floor areas from plant B (samples B1–B3) were chosen for the experimental testing. The floor areas were selected based on the observation that standing water was commonly observed during production. The floors consisted of concrete with epoxy coating (plant A) and polyurethane coating (plant B). Both plants performed daily cleaning with chlorinated alkaline based cleaning agents, followed by disinfection. Plant A used a quaternary ammonium compound based disinfectant for routine disinfection, while plant B used a disinfectant with peracetic acid as the active compound. The cleaning agents and disinfectants were used at the concentration recommended by the manufactures, with a minimum contact time of 10 min. Both plants rinsed with tap water (potable water) to remove disinfectants as the last step of their sanitation process.

Initially, the six sites were sampled for *L. monocytogenes* and total bacterial counts once a week for 4–5 weeks after cleaning and disinfection, but before production. Samples from floors/floor gutter (appr. 900 cm^2 surface area) were taken using neutralizing sampling cloths (Sodibox, Nevez, France). Analysis of *L. monocytogenes* was performed as described previously (Møretrø et al., 2017; NMKL, 2007) with preenrichment in half-Fraser broth (Oxoid, Basingstoke, UK) and enrichment in Fraser broth (Oxoid), followed by selective plating to RAPID'L.mono agar (Bio-rad), and confirmation of presumptive positive colonies by a *L. monocytogenes* specific PCR (Wesley et al., 2002). The samples from plant A were analysed at our laboratory, while samples from plant B were analysed by a commercial laboratory. Regarding total aerobic counts, samples were sent to laboratories and analysed the day after sampling by plating to TSA agar, with incubation at 20 °C for 3–5 days (plant A) and 30 °C for 2 days (plant B, performed by a commercial lab). In the test period, personnel from the processing plants were asked to pour 80–100 g of citric acid powder (Citric acid anhydrous, Weifang Ensign Industry Co., Ltd) once a day to areas up to 0.5 m^2, after the sanitation process but before start of production. At days with bacterial sampling (performed with sampling cloths as described above), sampling was performed before the addition of citric acid. For both plants sampling were performed weekly for four weeks, starting one week (plant A) and three days (plant B) after addition of citric acid started. For plant A, due to unforeseen practical circumstances there was a five weeks delay between the last sampling in the control period until addition of citric acid started.

Results and Discussion

The occurrence of *L. monocytogenes* was significantly lower ($p < 0.05$, Fisher's exact test) in the period when citric acid was added compared to the control period prior to citric acid addition (Table 1). In total, 3 out of 23 (13 %) samples from the test period with addition of citric acid were *L. monocytogenes* positive, compared to 16 of 27

TABLE 1: *Occurence of Listeria monocytogenes on floors before and in a period with addition of citric acid powder.*

Floor area	Samplings control period					Samplings citric acid period			
	1[a]	2	3	4	5	1[b]	2	3	4
A-1[c]	+[d,e]	–	+[e]	–	–	–	–	–	–
A-2	+[f]	+[g]	–	+[h]	+[h]	–	–		–
A-3	+[i]	–	–	+[e]	+[i]	–	–	–	–
B-1		–	+	+	–	–	–	–	–
B-2		+	+	+	–	–	–	–	–
B-3		+	+	–	–	–	+	+	+

[a]: Sampling in control period performed weekly for five and four weeks for plant A and B, respectively. [b]: Sampling in citric acid period performed weekly for four weeks, starting one week (plant A) and three days (plant B) after addition of citric acid started. For plant A, there was a five weeks delay between the last sampling in the control period until addition of citric acid started. [c]: Floor gutter, all other sample points were floors. [d]+/–: indicate sample positive/negative for *Listeria monocytogenes*. [e]: MLVA type 7-11-15-18-6, [f]: 6-0-14-10-6, [g]: 6-7-14-10-6, [h]: 8-7-0-7-11, [i]: 6-9-19-10-6. Clear cell indicates not sampled.

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(59 %) samples from the control period (Table 1). In the control period, 40–80 % of the samples from the individual sampling areas were positive for *L. monocytogenes,* despite daily conventional cleaning and disinfection. In the citric acid test period, all floor areas were negative for *L. monocytogenes,* with the exception of one floor in plant B that was positive in three out of four samplings. To our knowledge, there were no other major changes in routines in the plants besides the addition of citric acid in the test period compared to the control period. This supports the hypothesis that addition of citric acid powder led to control of *L. monocytogenes.* Typing of the *L. monocytogenes* isolates from plant A in another study, showed that the isolates in the control period were of several MLVA types, including three MLVA types (6-7-14-10-6, 6-9-19-10-6 and 7-11-15-18-6) that have previously repeatedly been isolated over time in plant A (Møretrø et al., 2017). This indicated that addition of citric acid aided to control *L. monocytogenes* types established in the processing plant. The analyses of samples from plant B were performed by a commercial laboratory, and as we did not get access to the isolates, they could not be typed.

TABLE 2: *Total aerobic counts (log_{10}) per sampling cloth on floors before and in a period with addition of citric acid powder.*

Floor area	Samplings control period					Samplings citric acid period			
	1[a]	2	3	4	5	1[b]	2	3	4
A-1[c]		>4.4[d]	>4.4	>4.4	>4.4	>4.4	>4.4	4.0	>4.4
A-2		>4.4	>4.4	>4.4	>4.4	>4.4	>4.4	>4.4	>4.4
A-3		>4.4	>4.4	>4.4	>4.4	>4.4	>4.4	2.7	3.6
B-1			>4.4	>4.4	>4.4	<1.0[e]	1.5	<1.0	<1.0
B-2			>4.4	>4.4	>4.4	>4.4	>4.4	<1.0	<1.0
B-3			>4.4	4.2	4.2	>4.4	2.8	>4.4	4.0

[a]: Sampling for total aerobic counts in control period performed weekly for four and three weeks for plant A and B, respectively. [b]: Sampling in citric acid period performed weekly for four weeks, starting one week (plant A) and three days (plant B) after addition of citric acid started. For plant A, there was a five weeks delay between the last sampling in the control period until addition of citric acid started. [c]: Floor gutter, all other sample points were floors. [d]: Above detection range. [e]: Under lower detection limit. Clear cell indicates not sampled.

Floor areas with standing water may act as sites where *L. monocytogenes* get established over time. *L. monocytogenes* has the potential to grow as food residues introduced during production may act as growth substrates. When adding citric acid powder to places where water is accumulating, the powder will dissolve and result in a low pH where *L. monocytogenes* cannot grow. The use of powder compared to fluid citric acid may result in a more long-term effect regarding lowering of pH, as the former will dissolve over time and not being rinsed or diluted away rapidly. Although one sanitation cycle may not totally eliminate *L. monocytogenes* and other bacteria in rough and wounded surfaces, repeating sanitation will reduce the number of bacteria over time when growth is not allowed between the sanitation periods. It has been suggested that the ability of *L. monocytogenes* to regrow between the daily sanitation cycles, is vital for its persistence in processing plants (Carpentier & Cerf, 2011). Citric acid is well known to have an antibacterial effect against *L. monocytogenes* and can be used as an ingredient in food or to decontaminate food, to control *L. monocytogenes* in food (Bal'a and Marshall, 1998; Gonzalez-Fandos et al., 2009; Over et al., 2009).

It is not clear why one of the floor areas of plant B (B-3) remained *Listeria*-positive in the citric acid period. As three out of four pH measurements in the citric acid period of this floor were in the area pH 6–8 (data not shown), it may be speculated that the amount of citric acid was too low due to large water volumes or high buffer capacity of the water. Another explanation may be that *L. monocytogenes* was frequently introduced to the B-3 floor area from another unknown reservoir in the production environment, as the floor area was commonly traffikked by personnel.

As procedural details for addition of citric acid were lacking we wanted to test the effect of a simple and robust procedure easy to perform for the industry. In the present study, 80–100 g of citric acid powder was added to areas of floors where water tend to accumulate during processing. The amount of citric acid powder added should be adjusted if the water volumes are large, or the buffer capacity is high. It may be checked with pH paper that the amount of powder added, results in a pH drop at least to pH 4.5, where *L. monocytogenes* do not grow (George et al., 1988). In plant B, pH was measured with pH paper and about half of the measurements were in the area pH 2–3. If citric acid powder is used, it should be checked that exposed floors and surfaces tolerate such low pH and do not deteriorate over time, as worn floors and surfaces may become harborage sites for *L. monocytogenes.* One possibility is to use the treatment as a response to *Listeria* positive samples or in predetermined periods to inhibit establishment of *L. monocytogenes,* e. g. for some weeks twice a year. Citric acid powder should be added daily in the test period, until the combined reduction due to growth inhibition due to citric acid and removal/killing by the sanitation process eradicate *L. monocytogenes.* The total bacterial counts were on average lower in the period with addition of citric acid than in the control period (Table 2). There was no statistic correlation between the total aerobic count and the occurrence of *L. monocytogenes* ($p>0.05$).

There is a need for novel control measures against *Listeria monocytogenes,* as the bacterium establishes itself in the food industry despite conventional cleaning and disinfection. In the present study an *in situ* evaluation of the use of citric acid powder against *L. monocytogenes* was performed. Regarding other control measures that have been tested *in situ* against *L. monocytogenes* on floors/drains, we are only aware of two studies where the addition of lactic acid bacteria has been shown to inhibit *L. monocytogenes* (Schöbitz et al., 2014; Zhao et al., 2013).

Conclusion

In conclusion, the present study documented that the advised addition of citric acid powder to floors may decrease the occurrence of *L. monocytogenes.* Such effect has not been previously reported in the scientific literature. The procedure for use of citric acid may have to be further optimized and adapted to each situation, but the procedure and results from the present study may be used as a starting point towards implementation in the food industry.

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Conflict of interest

The authors declare no conflicts of interest.

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