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EFFECT OF ACETIC, CITRIC AND LACTIC ACID ON *SALMONELLA ENTERITIDIS* AND *LISTERIA MONOCYTOGENES*

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Abstract

The influence of three organic acid, (acetic, lactic and citric acid in different concentrations) were evaluated over two major foodborne pathogens, *Listeria monocytogenes* and *Salmonella enteritidis*. For evaluation we used two different laboratory designed models such as, agar gel diffusion test and broth reduction test.

The results from agar gel diffusion tests revealed variation of the inhibition zone of *Listeria monocytogenes* and *Salmonella enteritidis* to three engaged organic acids in concentration 10%, 8%, 6%, 4%, 2%. The two tested organisms were most sensitive to lactic acid and slightly less sensitive to citric and acetic acid respectively. *Listeria monocytogenes* was more sensitive to all three tested organic acids compare to *Salmonella enteritidis*. Influence of above mentioned organic acids in buffered peptone water broths revealed *Listeria monocytogenes* reduction in cell number by 4,6 log cfu/mL for 0.04% acetic acid and 3,1 and 2.8 log cfu/mL for lactic acid and acetic acid respectively. Same test ravelled *Salmonella enteritidis* reduction in cell number by 3.8 log cfu/mL 0.04% for lactic acid and 2.9 and 2.5 log cfu/mL for citric and acetic acid.

Therefore these organic acids may have practical application in food processing industry for ensuring of food safety regarding to *Listeria monocytogenes* and *Salmonella enteritidis*.

***Key words**: Listeria monocytogenes, Salmonella enteritidis, organic acids*.

1. Introduction

Many food-borne pathogenic bacteria exhibit stress responses, which enhance their survival in adverse environmental conditions. One stress commonly encountered in foods is an acidic environment, where enhanced survival can involve induction of an acid tolerance response (ATR). The ATR is defined as the resistance of cells to low pH when they have been grown at moderately low pH or when they have been exposed to a low pH for some period of time (Dilworth and Glenn [1]). The mechanism of biocidal activity of organic acids is still unknown, but it was suggested that *Salmonella spp.* and *L. monocytogenes* does not have the ability to reduce intracellular pH, which causes a lethal accumulation of acid anions within the cell (Van Immerseel *et al*. [14]). *Listeria monocytogenes* and *Salmonella enteritidis* exhibit an ATR when exposed to mildly acidic pH (Foster and Hall [2]). The gram-positive bacterium *Listeria monocytogenes* is recognized as a food-borne pathogen with significance for humans (Farber and Peterkin [3]), and major outbreaks of infection have been linked to the consumption of contaminated coleslaw, cheeses (Fleming *et al*. [4]), and pasteurized milk (Ita and Hutkins [5]). Today, *L. monocytogenes* is a major concern to manufacturers worldwide due to the high mortality rate of listeriosis in susceptible populations and to the resistance of the pathogen to a number of food preservation practices. In particular, the ability of the organism to grow at refrigeration temperatures and on dry surfaces (Ahamad and Marth [6]) and its ability to tolerate acidic conditions (Conner *et al*. [7]) makes it well adapted to food environments which normally restrict bacterial growth. *Salmonella enteritidis* is an important pathogen causing foodborne infections in humans (Ahamad and Marth [6]). Poultry meat and eggs have been reported to be the major sources of *S. enteritidis* infection to humans. Numerous surveys conducted in various parts of the world indicate that *S. enteritidis* is the most common serotype of *Salmonella* isolated from poultry products (Ahamad and Marth [8]). Efforts to reduce the incidence of *S. enteritidis* in poultry meat and eggs will reduce the likelihood of foodborne outbreaks of this pathogen and decrease economic losses to the poultry industry (Vasudevan and Marek [9]). Consequently, control of these bacteria is a significant challenge for the food manufacturer. Acidification of foods with

short-chain organic acids, either by fermentation or by deliberate addition, is an important and widespread mechanism for controlling food-borne pathogens in a variety of foods. However, a number of studies have demonstrated that *L. monocytogenes* is more acid tolerant than most food-borne pathogens, although the sensitivity of the organism to organic acids varies with the nature of the acidulant used (Sorrells *et al*. [12]). An additional consideration relevant to the survival of this pathogen in foods is that fact that acid tolerance can be enhanced by exposing the organism to moderately acidic conditions (Davis *et al*. [10] and Kroll and Patchett [11]), a factor which can further reduce the effectiveness of acid-based preservation systems against *L. monocytogenes*. The innate resistance of *L. monocytogenes* to many of the food preservation systems that are effective against other foodborne pathogens has prompted research aimed at developing combination systems for more effective control of this pathogens (Oh and Marshall [13]).

2. Materials and Methods

Used reference strains of *Salmonella enteritidis* NCBB 100284 and *Listeria monocytogenes* NCCB 100286 were obtained from Food and Consumer Product Safety Authority (VWA), AL Groningen, (CHECK) The Netherlands. From each vial with reference cultures, 0.1 mL was transferred in test tube containing 10 mL of Buffered peptone water and incubated at 37°C for 24 hours. Bacterial numbers in obtained broths were calculated with preparation of decimal dilutions in Maximum recovery diluents and by plating out the nutrient agar using pour plate method. Bacterial count was expressed in cfu/mL and Log cfu/mL. Same broths were used for preparation of plates for agar gel diffusion-inhibition test, incorporating 0.1 mL of previously adjusted bacterial suspension with known cfu/mL in 1 mL semisolid nutrient broth containing 3 g of agar-agar per L. Over a plate (d = 90 mm) with previously solidified nutrient broth agar, to which 15 g of agar per liter were added, we pour 4 mL of semisolid nutrient broth, to with double less amount of added agar, containing required bacterial suspension ($2.1x10^5$ cfu/plate for *L. monocytogenes* and $3.2x10^5$ cfu/plate *S. enteritidis*). On the surface of the plates with sterile cylindrical instrument, wells with diameter of 10 mm were made and field immediately with 100 µL of the organic acid. Then, plates are left at room temperature for 2 hours and afterward put in to incubator at 37°C for 24 hours. The tests of inhibition in Maximum recovery dilluent (MRD) were carried out in test tubes containing 9 mL MRD and to which 1 mL of incubated broth culture were added. Then to each test tube with prepared bacterial suspension 1 mL of 0,04% tested organic acids was added. After 5, 10 15 and 20 minutes mixture of MRD, bacterial suspension and organic acid was neutralized with 0,1 nNaOH to neutral pH 7.0. To calculate log cfu/mL reduction of bacteria, after specified time we use 1, 0.1 and 0.01 mL of suspension and plate it out on Nutrient agar using pour plate method. Plates were incubated for 72 hours at 37°C and bacteria count was calculated and expressed in log cfu/mL.

3. Results and Discussion

Studies show that the zone of inhibition of growth in agar gel diffusion-inhibition test decreases slightly with the fall of the concentration of organic acids. Inhibition zone for *L. monocytogene*s was highest in lactic acid with 5 mm for 10% and 4 mm for 2% of the acid. Lactic acid has a lower zone of inhibition for *S. enteritidis* which ranges from 4 mm for 10% and 8% acid to 3,5 mm for 6% and 3 mm for 4 and 2%. The other two acids showed high zone of inhibition of *L. monocytogenes* with range for citric acid of 4,5 for 10%, mm 4 mm for 8 and 6% and 3,5 mm for 4% and 3 mm for 2%. Acetic acid had a slightly smaller zone of inhibition 3,5 mm for 6%, amounting to as much as 3 mm zone of inhibition for 2% acid. Assessment of the influence of citric and acetic acid on *S. enteritidis* had slightly weaker effect of inhibition zone that ranges from 3mm for highest to 2 mm for the lowest concentrations of acid. Graphic results of inhibition from agar gel diffusion-inhibition test for zone of inhibition are presented in Figure 1.

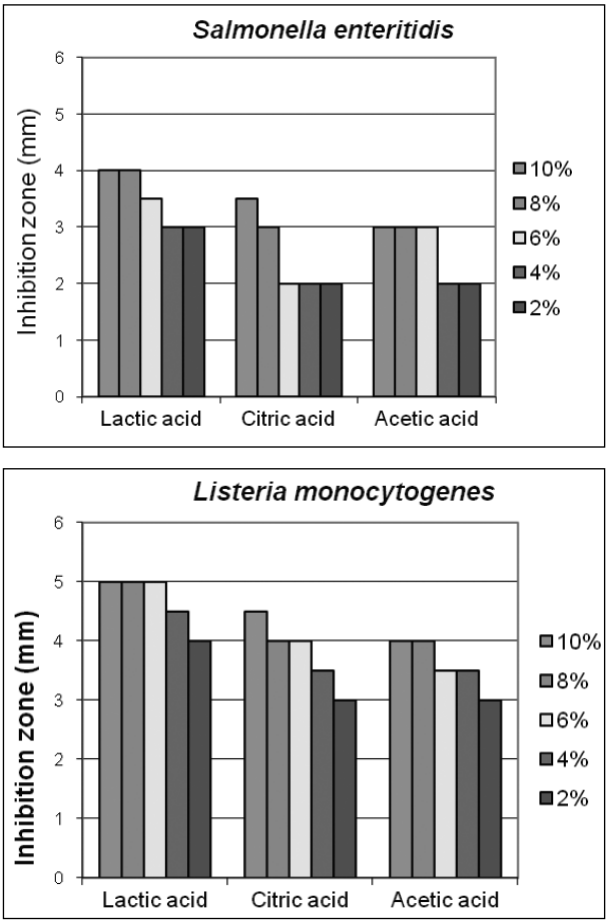

Figure 1. Zone of growth-inhibition on nutrient agar plates expressed in mm from the edge of the well

filed with 0,1 mL of tested organic acid in different concentration 10, 8, 6, 4 and 2%. Tested plate is covered with bacterial suspension in semi-solid nutrient agar containing 2.1x10^5 cfu/plate for *L. monocytogenes* and 3.2x10^5 cfu/plate *S. enteritidis*

Test of inhibition in MRD broth revealed decreasing of the number of bacteria by increasing the concentration of organic acid and prolonging time until the neutralization of the acid. The highest decline in log cfu/mL for lactic acid has *L. monocytogenes* 4,6 log cfu/mL, while *S. enteritidis* has decreased by 3,8 log cfu/mL in 20 minutes. Citric and acetic acid reduced the number of *L. monocytogenes* in the first 5 minutes for 0.8 and 1,4 log cfu/mL. The number of *S. enteritidis* in the same period for two acids is reduced by 0.7 to 1 log cfu/mL. It was notable that the rapid decline in the number of bacteria of *L. monocytogenes* in the first 5 minutes compared with *S. enteritidis* where decline in bacterial count was milder.

Results from the reduction of bacteria in MRD are presented in Figure 2.

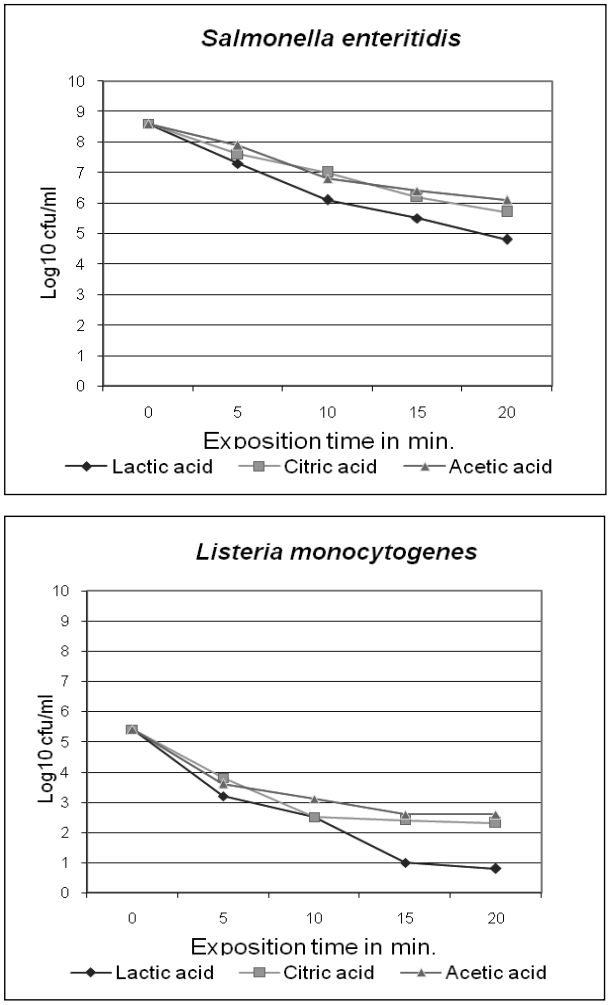

Figure 2. Reduction of bacteria expressed in log cfu/mL MRD broth, measured after exposition of working suspension at 5, 10, 15, 20 minutes, immediately after neutralization with 0.1nNaOH at pH 7,0. Exposition time of 0 minutes represents the number of bacteria in log cfu/mL in MRD suspension before adding 1 mL of 0,04% organic acid

4. Conclusions

- The results of this study indicates that organic acids have inhibitory effect on growth and contribute to reduction in number of two tested major food-borne pathogens, L. monocytogenes and S. enteritidis in in-vitro laboratory designed models. Differences in reactions between short-chain organic acids may be explained by the differing ability to alter the pH of the cell. Certain organic acids may enter into the cell more easily than others and therefore alter the pH of the cell more readily. Acetic acid is lipid soluble, diffuses rapidly through the plasma membrane and has been shown to have a dramatic effect on the pH of a cell (Dilworth and Glenn [1]). In contrast, lactic acid demonstrates some lipid solubility and diffuses slowly through the membrane. Disruption of the cell pH does not appear to be its main mode of inhibition (Dilworth and Glenn [1]). If the pH of the cell is reduced more readily with acetic acid than with lactic acid, then the optimum adaptation time for acetic acid may also be reduced, and the optimum adaptation time may differ according to the organic acid used in the examination.
- Differences observed between organisms may result from differences in membrane structure. Previous studies have shown that inorganic acid adaptation provides protection against organic acid stress and vice versa (Foster and Hall [2] and Vasudevan and Marek [9]).
- Influence of tested organic acids over inhibitory effect on growth and reduction in number shows that lactic acid had highest effect over the microorganisms, comparing with two other tested acids, citric and acetic. These two in-vitro different laboratory designed methods gave evidence for influence of short-chain organic acids over inhibition zone and reduction number of L. monocytogenes and S. enteritidis.

5. References

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