https://doi.org/10.17113/ftb.64.02.26.9340 original scientific paper

Organic Acid Meat Decontamination: Optimizing Application Parameters to Reduce the Microbial Load of *Yersinia enterocolitica* 4/O:3 on Pork

Running head: Preservation of Pork Following Organic Acid Decontamination

Marta Kiš[1*], Jasenka Gajdoš Kljusurić[2] and Nevijo Zdolec[1]

[1]University of Zagreb, Faculty of Veterinary Medicine, Hygiene, Technology and Food Safety Unit, Heinzelova 55, Zagreb, Croatia
[2]University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Process Engineering, Pierottijeva 6, Zagreb, Croatia

Received: 28 August 2025
Accepted: 7 December 2025

SUMMARY

Research background. Pigs are natural carriers of pathogenic bioserotypes of *Yersinia enterocolitica* and the consumption of undercooked pork is a risk factor in the epidemiology of yersiniosis. The aim of this study was to determine the decontamination effect of lactic and acetic acid on pork cuts inoculated with *Yersinia enterocolitica* 4/O:3 strains (N=10) under laboratory conditions and to compare their effect with the one obtained after water washing.

Experimental approach. A total of 20 decontamination protocols were carried out in which the effect of organic acid solutions (2 and 4 %) and water was investigated at different temperatures (25 and 80 °C) and exposure times (spraying for 10 and 30 s) in two time intervals (0 and 24 h).

Results and conclusions. The decontamination effect obtained after application of lactic acid solutions was significantly higher ($p<0.05$) than that of acetic acid and water immediately after the

---

[*]Corresponding author:
Phone: +38512390199
E-mail: mkis@vef.hr

treatment. After 24 h, their effect equalises ($p>0.05$), which can be attributed to the residual effect of the acids and the inadequate response of the cells to the cold conditions in the case of water washing. The factor analysis showed that hot solutions applied for a longer exposure time had the greatest influence on the reduction of *Y. enterocolitica* 4/O:3 counts ($p<0.05$) in contrast to the type of acid and its concentration ($p>0.05$).

Novelty and scientific contribution. This is the first organic acid susceptibility study focusing on the pathogenic bioserotype 4/O:3 of *Y. enterocolitica.* The study provides valuable insights for the development of strategies to control pathogenic *Y. enterocolitica* in the pork production chain and serves as a basis for future research.

Keywords: *Yersinia enterocolitica* 4/O:3; pork; organic acids; water; decontamination

INTRODUCTION

Microbiological status of animal carcasses and of portioned meat is one of the basic requirements for ensuring hygienically safe raw products. In connection with microbiological safety, the focus is primarily on pathogenic bacteria such as *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* and *Escherichia coli*, the presence of which is largely determined by the animal species, the meat. For example, *Salmonella* spp. and *Y. enterocolitica* are listed as priority biological hazards in the control of pork due to their presence in pigs, confirmed cases of human disease and epidemiological studies (*1*). In contrast to *Salmonella* spp., most countries still do not have national control programs for *Y. enterocolitica*, although the number of reported cases of yersiniosis is increasing (*2*).

Pigs are among the most common asymptomatic carriers of pathogenic strains of this bacterium, which mainly colonize the tonsils, lymph nodes and intestines (*3-5*). Serotypes O:3, O:8, O:9, and O:5,27 are most frequently associated with human infections (*6,7*), and according to EFSA and ECDC (*2*), the most common bioserotypes in Europe are 4/O:3 (86.9 %) and 2/O:9 (10.7 %). In many countries, bioserotype 4/O:3 remains by far the most frequently isolated from pigs (*8-11*), indicating the significant role of pigs in the epidemiology of human yersiniosis. The link between consumption of contaminated pork products and yersiniosis has been documented in several studies from different countries (*12-14*).

In this context, the processing of pig carcasses in the slaughterhouse is considered to be the most important for preventing contamination of the meat and thus the entry of *Y. enterocolitica* into the further pork production chain. The standard intervention methods, which have certainly contributed to a lower prevalence of *Y. enterocolitica* on the meat, mostly included procedures such as bagging of the rectum, omitting the incision of lymph nodes at veterinary *post mortem* meat inspection and separation of the tonsils from the rest of the carcass. However, these measures are usually not sufficient to completely eliminate the risk, as the pathogen is very common in the tonsils (*15*) and is psychrotrophic. This was confirmed by a recent study in Croatia (*16*), in which the pathogenic *Y. enterocolitica* 4/O:3 was found in 101 of 234 (43.16 %) samples of pig tonsils examined.

The use of organic acids in the decontamination of carcasses and meat cuts has been the subject of numerous studies for many years (*17-19*) and is considered as a way to improve the hygienic condition of the carcasses and thus the safety of the meat, taking into account the antimicrobial effect on pathogenic microorganisms (*20*). However, it is important to emphasise that decontamination methods must be part of an integrated food safety system and are in no way a substitute for good hygiene or manufacturing practises. Lactic acid and acetic acid are among the most studied organic acids to date (*18,21,22*), as they are widely distributed in nature but also used in the food industry (*23*). With regard to other pathogenic bacteria in meat hygiene, the literature review revealed a lack of data on the decontamination effect of organic acids against the *Y. enterocolitica* population on pork (*24*) and on the comparison of their efficacy with conventional washingmethods using potable water.

Potable water is currently the only decontamination agent authorised in the EU for slaughtered carcasses, at least for pigs. The use of all other potential substances for decontamination purposes requires prior authorisation by the European Commission. Taking into account the EFSA Scientific Opinion (*25*), which reviews the decontamination potential of organic acids in reducing microbiological surface contamination of pig carcasses using the example of cattle carcasses (*26*), the aim of this study was to evaluate the decontamination effect of lactic and acetic acid on pork inoculated with different strains of *Y. enterocolitica* 4/O:3 and to compare their effect with the one obtained after water washingunder the same conditions. The use of different concentrations and temperatures of the solutions used, as well as the duration of the decontamination protocol, will provide information on the optimal protocol that leads to the most desirable reductions in bacterial counts. Due to the complexity of the research methodology to be carried out on the slaughter line, the entire study was conducted under laboratory conditions.

MATERIALS AND METHODS

Experiment plan

Based on the selected factors (type of organic acid, acid concentration, acid solution temperature and exposure time), a factorial experimental design was established to determine the minimum number of protocols per *Y. enterocolitica* 4/O:3 strain. Considering four conditions with two levels each, a 2×2×2×2 design was used, resulting in a total of 16 protocols. A further 4 were added with water, at two temperatures and two exposure times, bringing the total number of protocols to 20 (Table 1). The verification of the reduction in the number of *Y. enterocolitica* 4/O:3 was carried out in two measurements (0 and 24 h) in triplicate.

Preparation of Y. enterocolitica 4/O:3 strains

Y. enterocolitica 4/O:3 strains (*N*=10) used for the experiment were collected as part of a previous study (*16*) and stored in a microbank cryopreservation system (Deltalab, Barcelona, Spain) at -80 °C until testing. Immediately prior to inoculation on meat, the strains were multiplied in PSB broth (peptone, sorbitol and bile salts, PSB, Sigma Aldrich, St. Louis, USA), which was then serially diluted to obtain optimal dilutions for meat contamination. Each strain was treated separately with 20 different protocols.

Preparation of organic acids solutions

The decontamination solutions were prepared by diluting liquid concentrated (90–99.5 %) lactic acid and acetic acid (Merck, Darmstadt, Germany) with distilled water (Merck, Darmstadt, Germany). For the preparation of 2 and 4 % solutions, the required volumes of acids and water were calculated according to the following formula:

$$V(\text{initial volume}) = \frac{V\ (\text{final volume})\ x\ c\ (\text{final concentration})}{c\ (\text{initial concentration})} \qquad /1/$$

The pH of final solutions was measured prior to decontamination (pH meter, Testo, Lenzkirch, Germany).

Meat decontamination using organic acid solutions

Decontamination was performed on fresh retail pork (*M. longissimus dorsi*), without skin or fat. For each protocol, a piece of meat was used with an average mass of 100 g, of the same size and thickness, on which the pH was measured before inoculation of *Y. enterocolitica* 4/O:3 strains. The meat was tempered to room temperature before inoculation. PSB broth containing multiplied *Y. enterocolitica* 4/O:3 strains was serially diluted 1:9 after 24 h of incubation at 30 °C, and 1 mL of the appropriate dilution was inoculated evenly over the meat surface with a sterile wand, achieving an average meat contamination of 4 log CFU/g (control). To simulate the contamination conditions in the slaughterhouse, the decontamination protocols were started 5 min after bacterial inoculation on the meat (cell adhesion). Water and prepared 2 and 4 % solutions of organic acids were applied at different temperatures (25 and 80 °C) and exposure times (10 and 30 s) to previously contaminated meat by spraying it with an automatic sprayer (Gloria Haus und Garten, Gütersloh, Germany) at a pressure of 3 bar at a distance of 10 cm. Five min after inoculation and before the start of decontamination, the meat was cut into two equal parts, one half serving as a positive control and the other half hung on a suitable rack for decontamination. Using a gravimetric diluter (DiluFlow, Interscience, Cantal, France), the samples were weighed into a sterile bag into which saline solution was then added at a ratio of 1:10. After homogenization for 1 min (BagMixer, Interscience, Cantal, France), 0.1 mL was superficially inoculated onto CIN selective agar (Cefsulodin, Irgasan^{TM}, Novobiocin, Sigma Aldrich, St. Louis, USA) according to the reference method. After incubation for 24 h at 30 °C, the number of grown colonies was determined using an automatic colony counter (Scan 1200, Interscience, Cantal, France) and the result was expressed as log CFU/g. The same procedure was carried out on refrigerated samples after 24 h. The logarithmic values of the *Y. enterocolitica* 4/O:3 number after a given decontamination protocol were subtracted from the values of the positive controls and are presented as the reduction in the *Y. enterocolitica* 4/O:3 number per gram of a meat (R; $\log_{10}$). In addition, to exclude the possible presence of *Y. enterocolitica* in the meat due to contamination during production, a microbiological examination of a sample was performed on each individual meat package using the method described above. In order to determine the changes in the meat surface pH after decontamination, the pH of the solvent of the control and test samples was measured after homogenization of each individual sample was completed. All measurements were performed in triplicate and the results were expressed as mean value±S.D.

Statistical analysis

The results were processed using descriptive statistical methods (Statistica 13.5, TIBCO software) (*27*) and presented as mean values of three measurements with standard deviation (x±S.D.). To determine statistically significant differences between the quantitative data, Student's t-test was used for indicators that followed a normal distribution and Mann-Whitney's U-test was used for indicators that did not follow a normal distribution. The normality of the distribution was checked using the Kolmogorov-Smirnov test, with a p-value of 0.05. The correlation between the decrease in meat pH and bacterial reduction was tested using the correlation test (r). Multivariate analysis tools were used for qualitative data processing. Factorial analysis of variance (factorial ANOVA) was used to examine the influence of the independent variables (factors) on the dependent variable (reduction in the number of *Y. enterocolitica* 4/O:3). Statistical significance was determined at the 0.05 level.

RESULTS AND DISSCUSION

Decontamination with acidic solutions reduced the inoculated population of *Y. enterocolitica* 4/O:3 in all combinations of acid concentration, solution temperature, and decontamination duration. Their effect mostly depends on application temperature and exposure duration ($p<0.05$), in contrast to the type of acid and its concentration ($p>0.05$). Although lactic acid solutions produced the strongest initial decontamination effect, their impact after 24 h was equal to that of acetic acid solution and water.

Previous research on chemical decontamination of pork has mostly focussed on the use of lactic acid solutions (*28-31*) or a combination with acetic acid (*32,33*). The investigation of decontamination effect in such and similar studies is aimed at reducing the overall microbiological contamination in terms of the total population of bacteria or enterobacteria, while in the case of pathogenic bacteria, the focus is on *Salmonella* spp., which makes a comparison of the results obtained in relation to *Y. enterocolitica* difficult to some extent. Although some authors (*34,35*) point out that *Y. enterocolitica* is generally sensitive to the effect of organic acids, our results of the main reductions (Table 2) indicate that the strains of *Y. enterocolitica* 4/O:3 are more resistant to the effect of organic acids compared to the results of other studies (*28,32,36*). However, when comparing such results, all parameters used in the experiment that may have influenced the reduction in bacterial load must be taken into account. These are primarily the type of acid, its concentration, the temperature and method of application, the duration of exposure, the initial number of inoculated bacterial cells as well as the combinations of these variables, so a comparison of these results requires a critical approach (*37*).

Comparing the differences between the decontamination protocols using different types of organic acids, lactic acid was found to be significantly more effective ($p<0.05$) in all cases except when using 2 % hot acid solutions for 10 s and 4 % hot acid solutions for 30 s (Table 2). In these protocols the reduction was also higher when using lactic acid, but without statistical significance ($p>0.05$). This is consistent with the results of most previous studies on a similar topic (*23,38-41*), in which lactic acid had a better decontamination effect compared to acetic acid. Although both lactic and acetic acids in their undissociated forms have a stronger bactericidal or bacteriostatic effect than inorganic acids, there is a difference in antimicrobial properties between the two. This is defined as the specific effect of the acid, which is known to be most pronounced in lactic acid (*35*), supporting our results. However, the above-mentioned differences between the observed protocols were not significant even after 24 h ($p>0.05$), which is mainly due to the increase in reduction values observed after acetic acid treatment (Table 3).

The reason for this can be attributed to the stronger residual effect of acetic acid on meat, which, despite its weaker effect immediately after the treatment, considerably impedes the growth and survival of the remaining live, but damaged bacterial cells, especially when the growth is compared with untreated bacterial cells from control samples. The same conclusions were reached by Dan *et al.* (*32*), who found 3 %-5 % acetic acid solutions to be more effective in reducing *Yersinia* spp. counts. In this respect, no significant differences were found in the case of lactic acid due to the initially better antimicrobial effect at 0 h, which, however, supports the previously mentioned conclusions of most authors.

Namely, there was no statistically significant difference ($p>0.05$) in the reduction of pathogens using different concentrations of acetic or lactic acid solutions at the same exposure time and solution temperature. The same results were recorded after 24 h as well. Regarding the concentration of acids used, although numerous studies have shown that their increase correlates with a reduction in the bacterial count (*40,42-44*), in our case, the use of 4 % solutions of both types of acid did not lead to a statistically higher reduction in the *Y. enterocolitica* 4/O:3 population. However, some authors (*45,46*) have come to the same conclusion when using an even wider range of acid concentrations. Considering the pH values of such solutions or the small variations between the concentrations used (0.15-0.18), it can be expected that 4 % acid solutions are not sufficient to induce a stronger antimicrobial effect in most cases, at least not to the same extent as other observed parameters.

Thus, comparing the decrease in the meat pH after decontamination with the reduction levels of *Y. enterocolitica* 4/O:3 populations, it was found that the decrease in pH correlated moderately with the degree of reduction (Fig. 1), *i.e.* a higher decrease in meat pH led to a higher reduction in the *Y. enterocolitica* 4/O:3 population ($r=0.69$, $p<0.01$). A linear regression model confirmed this relationship, showing that approximately 48 % of the variation in bacterial reduction can be explained by the pH decrease ($R^2=0.48$). Thus, viewed individually, it was found that in more than 50 % of protocols, a statistically significant decrease in the pH of decontaminated meat did not lead to a statistically significant reduction in the inoculated population of *Y. enterocolitica* 4/O:3, supporting the fact of the insignificance of the acid solution concentration. This is best illustrated by the example of aggregate pH values of meat measured after 24 h of cooling, where, under the influence of strong buffer capacity, the pH returns to physiological levels (data not shown), while a simultaneous decline in the population of inoculated bacterial cells is observed. The above supports the consideration of other potential negative consequences of decontamination, such as the effects on the organoleptic properties of meat, especially in the case of acetic acid, where even a small increase in concentration can have negative effects on meat odour.

When temperature was considered as a factor, a significantly higher ($p<0.05$) reduction was achieved when hot acid solutions were used, but only during a longer exposure time of 30 s. In this respect, exposure over a period of 30 s significantly ($p<0.05$) influenced a higher reduction in the bacterial population compared to a shorter exposure of 10 s, but only in the case when hot solutions were used. This was confirmed by a factorial analysis (Table 4) in which the use of acidic solutions at different temperatures with shorter or longer exposure times proved to be a more significant factor ($p<0.05$) in reducing bacterial populations, with higher temperatures and longer decontamination protocols being mutually dependent.

The literature contains contradictory information on these factors, which in turn can be attributed to the use of different experimental methods, making their interpretation difficult. For example, Christiansen *et al.* (*28*) found a significantly higher reduction in the number of *Salmonella* spp. when a 2.5 % lactic acid solution was applied for 5 s longer, whereas in the study by Laury *et al.* (*47*) even a 15 s longer application of the same type and concentration of acid had no effect on the differences in their numbers. The above differences are actually to be expected, especially considering the other parameters that differed, such as the type of raw material treated (pork steaks; chicken carcasses with skin), the method of treatment (acid spraying, immersion in the solution), the temperature of the solution used (55-80 °C; room temperature) and the number of bacterial cells inoculated (10^7 CFU/cm^2; 10^6 CFU/mL). The complexity of comparing such results is further illustrated in the aforementioned study by Christiansen *et al*. (*28*), in which a better decontamination effect was achieved through longer treatment and higher temperature of the applied solutions, despite some parameters favouring reduced sensitivity or a weaker inhibitory effect. For example, the concentration of the lactic acid solution was lower compared to ours, the exposure time was shorter, the adhesion time of the bacteria was significantly longer, and the concentration of bacteria per cm^2 was higher, with the note that, in addition to *Y. enterocolitica*, *Salmonella* spp. and *E. coli* were also inoculated. The latter may explain the increased reduction due to the weak competitiveness of *Y. enterocolitica*. However, drawing any conclusions requires data with clearly equal parameters, which are far from sufficient in the case of *Yersinia*. Our results showed a slightly higher reduction in the number of *Y. enterocolitica* 4/O:3 in all protocols with prolonged exposure, but as we have already mentioned, only the reduction using hot acidic solutions were statistically significantly higher. One possible explanation for this lies in the temperature of the solution, which was heated to 80 °C immediately before application, but cools down quickly during spraying and thus has a lower efficiency, which is then compensated for by a longer application of 30 s. As there were no such significant differences when cold solutions were used, the ones observed are probably due to mechanical influences or the "rinsing effect".

Guided by the recommendations of the EFSA Scientific Opinion (*25*), which point out the lack of scientific evidence comparing the decontamination effect of lactic acid and water as the only authorised decontaminant in pigs, another objective of this study was to determine whether the application of water under the same conditions leads to an equivalent reduction in the number of *Y. enterocolitica* 4/O:3 on meat. Washing (spraying) carcasses with water is a routine procedure on the slaughter line that has been proven to reduce the present microorganisms (*20*). The water wash effectiveness has been observed in research mostly in connection with the inclusion of water as an additional agent in decontamination protocols with organic acids, and usually such combinations led to the strongest reductions in the target bacterial population (*31,48,49*).

Comparisons of identical decontamination protocols (in terms of temperature and duration) with acetic acid, lactic acid and water are shown in Table 5. In contrast to acetic acid, protocols with lactic acid showed a significantly higher efficacy in reducing the inoculated population of *Y. enterocolitica* 4/O:3 on meat compared to standard protocols with water. A statistically higher reduction ($p<0.05$) at 0 h was observed in all protocols with lactic acid compared to water, except in protocol no. 13. Surprisingly, compared to 0 h, after 24 h there were no significant differences in the reduction of *Y. enterocolitica* 4/O:3 count in decontaminated meat between the observed protocols ($p>0.05$).

The comparison of two measurement intervals (0 and 24 h) within the test and control samples clearly shows that the use of decontamination protocols with organic acid solutions, in addition to the initial reduction in the number of *Y. enterocolitica* 4/O:3, also results in the further reduction of the population during meat chilling (Fig. 2). This was also observed in the control samples, but to a lesser extent and without statistical significance. This is not surprising considering the psychrotrophic nature of *Y. enterocolitica*, which allows it to survive under chilling conditions, so that its numbers are maintained within a stable population with only minor variations. On the other hand, in the treated samples immediately after exposure, the inoculated bacterial population decreases and the number of remaining surviving but damaged bacterial cells stagnates and decreases over time. The reason for this is the aforementioned residual effect of organic acids, which, according to Rodriguez-Melcon *et al.* (*46*), persists in the case of lactic acid even 120 h after its application and thus leads to an additional reduction in the number of the bacterial population (>2 log) compared to the control sample. This explains the stronger reductions achieved in our study, which, despite the initially stronger effect of lactic acid, led to an equalisation of its effect with that of acetic acid (Table 6).

As for the water, given that in this case there was no residual influence of acids or low pH, it is difficult to determine with certainty the reason for the above results. In view of the fact that the differences between the control and test samples indicate a different development in the number of bacterial cells, it is understandable to consider the role of water application in this context. Indeed, after the application of water, the meat is rapidly cooled to 4 °C, with the water remaining on the surface contributing to accelerated cooling and exposure of the bacterial cells to the so-called "cold reaction". As numerous authors have noted (*50-52*), the ability to adapt to such temperature differences must be accompanied by a series of complex biochemical and cellular changes in the bacterial cell, such as membrane permeability, protein conformation or gene expression. In the study by Li *et al.* (*53*), the physiological processes of the cold response in *Y. enterocolitica* were investigated by comparing growth ability, gene and protein expression and cell motility after exposure to cold conditions. The induction of the cold response was performed in 55 strains of *Y. enterocolitica* of different bioserotypes, and the results showed, among other things, that the degree of induction of such a response is strain-specific and temporally conditioned by adaptation to cold conditions. Relating the above statements to our studies, it is possible that the decrease in the number of *Y. enterocolitica* 4/O:3 after 24 h of refrigeration is due to their low adaptation to the cold conditions, especially since such a tendency was not present in the untreated control samples.

The relatively low reduction of *Y. enterocolitica* 4/O:3 count achieved in this type of research, *i.e.* under laboratory conditions, is not necessarily to be expected when this type of research is carried out under industrial conditions, *i.e.* on the slaughter line. Indeed, the conditions on the slaughter line differ considerably from those in the laboratory, starting with the meat as a treatment medium, the microclimatic conditions to which the meat is exposed, but also the decontamination process itself. Firstly, the concentrations of 10^3 or 10^4 per gramme or cm^2 used in such studies are unlikely to occur at the slaughter line, severely obstructing the survival of *Yersinia*, which is otherwise not very competitive, especially compared to other psychrotrophic bacteria. It is assumed that the contamination of carcasses with *Y. enterocolitica* at the slaughter line is less than 1 log CFU/cm^2 (*54,55*), apart from the fact that these cells are not adapted to acidic conditions. In this respect, the bacterial cells used in laboratory tests are generally in a stationary growth phase during inoculation, which enables them to adhere better and thus react less sensitively to acid than would be the case with contamination under natural conditions (*56*). Furthermore, decontamination at the slaughter line is usually carried out on carcasses that are still warm, which shows a better decontamination effect than with chilled meat (*35*). The overgrowth of muscle with fatty tissue (which was not present in the laboratory meat sample) contributes to a better decontamination effect due to a weaker buffering capacity compared to meat (*57*). In addition, the refrigeration conditions to which meat is exposed in the laboratory differ considerably from those in industry, where strong air currents cause a sudden drop in water activity, *i.e.* surface drying, which contributes to the reduction of surviving but damaged cells. Finally, under industrial conditions, the decontamination parameters themselves would be fulfilled with the application of a higher spray pressure in combination with an additional rinse with water.

However, when considering decontamination of the entire carcass and to predict the above-mentioned facts, it is necessary to conduct the same study on a skin model, which is more exposed to contamination on the slaughter line.

CONCLUSIONS

Considering all the results of this work, the decontamination potential of both types of acids in reducing the number of *Y. enterocolitica* 4/O:3 on pork is evident. The effect depends on the type of decontamination protocol, in particular the temperature of the solution and the duration of exposure. Regarding the type of acid, a better decontamination effect was obtained with lactic acid, and the reduction was higher when hot solutions of longer exposure time were used. However, after 24 h it equalises with the effect after using acetic acid solution and water. This is the result of the growth stagnation of the untreated population of bacterial cells and the simultaneous decrease in the number of exposed cells. In the first case we attribute this to the residual effect of the acids, while in the second case to the inadequate response of the cells to the cold conditions to which they are exposed immediately after exposure. Overall, our study contributes valuable insights into the development of strategies to control pathogenic *Y. enterocolitica* in the pork production chain and serves as a basis for future research. In addition, further studies are needed to assess the long-term impact of such methods on bacterial survival regarding the development of resistance to acids or the induction of a non-culturable state.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

M. Kiš prepared the study design, carried out the laboratory analysis and statistical evaluation of the results and wrote the manuscript. N. Zdolec supervised the laboratory analysis, reviewed and edited the manuscript. J. Gajdoš Kljusurić performed the statistical analysis of the results and reviewed the manuscript.

ORCID ID

M. Kiš https://orcid.org/0009-0002-0439-7829

J. Gajdoš Kljusurić https://orcid.org/0000-0001-6657-7337

N. Zdolec https://orcid.org/0000-0001-7413-8115

REFERENCES

1. Alvseike O, Prieto M, Torkveen, K, Ruud C, Nesbakken, T. Meat inspection and hygiene in a Meat Factory Cell – An alternative concept. Food Control. 2018;90:32-9. https://doi.org/10.1016/j.foodcont.2018.02.014

2. European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC). The European Union One Health 2023 Zoonoses Report. EFSA J. 2024;22: 1-201. https://doi.org/10.2903/j.efsa.2024.9106

3. Fois F, Piras F, Torpdhl M, Mazza R, Ladu D, Consolati SG, Spanu C, Scarano C, De Santis EP. Prevalence, bioserotyping and antibiotic resistance of pathogenic *Yersinia enterocolitica* detected in pigs at slaughter in Sardinia. Int J Food Microbiol. 2018;283:1-6. https://doi.org/10.1016/j.ijfoodmicro.2018.06.010

4. Angelovska M, Zaharieva MM, Dimitrova LL, Dimova T, Gotova I, Urshev Z, Ilieva Y, Kaleva MD, Kim, TC, Naydenska S, Dimitrov Z, Najdenski H. Prevalence, genetic homogeneity, and antibiotic resistance of pathogenic *Yersinia enterocolitica* strains isolated from slaughtered pigs in Bulgaria. Antibiotics. 2023;12:716. https://doi.org/10.3390/antibiotics12040716

5. Yue Y, Zheng J, Sheng M, Liu X, Hao Q, Zhang S, Xu S, Liu Z, Hou X, Jing H. Public health implications of *Yersinia enterocolitica* investigation: an ecological modeling and molecular epidemiology study. Infect Dis Poverty. 2023;12:1-15. https://doi.org/10.1186/s40249-023-01063-6

6. Bottone EJ. *Yersinia enterocolitica*: Revisitation of an enduring human pathogen. Clin Microbiol Newsl. 2015;37:1-8. https://doi.org/10.1016/j.clinmicnews.2014.12.003

7. Shoaib M, Shehzad A, Raza H, Niazi S, Khan IM, Akhtar W, Safdare W, Wang Z. A comprehensive review on the prevalence, pathogenesis and detection of *Yersinia enterocolitica*. RSC Adv. 2019;9:41010-41021. https://doi.org/10.1039%2Fc9ra06988g

8. Råsbäck T, Rosendal T, Stampe M, Sannö A, Aspán A, Järnevi K, Lahti ET. Prevalence of human pathogenic *Yersinia enterocolitica* in Swedish pig farms. Acta Vet Scand. 2018;60:39. https://doi.org/10.1186/s13028-018-0393-5

9. Sacchini L, Garofolo G, di Serafino G, Marotta F, Ricci L, di Donato G, Miracco MG, Perletta F, di Giannatale E. The prevalence, characterisation, and antimicrobial resistance of *Yersinia enterocolitica* in pigs from Central Italy. Vet Ital. 2018;54:115-23. https://doi.org/10.12834/VetIt.1126.6109.2

10. Koskinen J, Keto-Timonen R, Virtanen S, Vilar MJ, Korkeala H. Prevalence and Dynamics of Pathogenic *Yersinia enterocolitica* 4/O:3 Among Finnish Piglets, Fattening Pigs and Sows. FPD. 2019;16:12. https://doi.org/10.1089/fpd.2019.2632

11. Terentjeva M, Ķibilds J, Gradovska S, Alksne L, Streikiša M, Meistere I, Valcina O. Prevalence, virulence determinants, and genetic diversity in *Yersinia enterocolitica* isolated from slaughtered pigs and pig carcasses. Int J Food Microbiol. 2022;376:109756. https://doi.org/10.1016/j.ijfoodmicro.2022.109756

12. Almeida AMP, Leal NC. Advances in Yersinia Research. Book of Abstracts of the 10th International Symposium; 2010 October 23 – 27; Recife, Brasil; pp. 367.

13. Le Guern AS, Martin L, Savin C, Carniel E. Yersiniosis in France: overview and potential sources of infection. Int. J. Infect. Dis. 2016;46:1-7. https://doi.org/10.1016/j.ijid.2016.03.008

14. Guillier L, Fravalo P, Leclercq A, Thébault A, Kooh P, Cadavez V, Gonzales-Barron U. Risk factors for sporadic *Yersinia enterocolitica* infections: a systematic review and meta-analysis. Microb. Risk Anal. 2021;100141. https://doi.org/10.1016/j.mran.2020.100141

15. Zdolec N, Kiš M. Meat Safety from farm to slaughter—risk-based control of *Yersinia enterocolitica* and *Toxoplasma gondii*. Processes. 2021;9:815. https://doi.org/10.3390/pr9050815

16. Zdolec N, Kiš M, Jankuloski D, Blagoevska K, Kazazić S, Pavlak M, Blagojević B, Antić D, Fredriksson-ahomaa M, Pažin V. Prevalence and persistence of multidrug-resistant *Yersinia enterocolitica* 4/O:3 in tonsils of slaughter pigs from different housing systems in Croatia. Foods. 2022a;11:1459. https://doi.org/10.3390/foods11101459

17. Stivarius M, Pohlman RFW, Mcelyea KS, Waldroup AL. Effects of hot water and lactic acid treatment of beef trimmings prior to grinding on microbial, instrumental color and sensory properties of ground beef during display. Meat Sci. 2002;60:327-34.
https://doi.org/10.1016/S0309-1740(01)00127-9

18. Theron MM, Lues JFR. Organic acids and meat preservation: a review. Food Rev Int. 2007;23:141-58.
https://doi.org/10.1080/87559120701224964

19. Han J, Luo X, Zhang Y, Zhu L, Mao Y, Dong P, Yang X, Liang R, Hopkins DL, Zhang Y. Effects of spraying lactic acid and peroxyacetic acid on the bacterial decontamination and bacterial composition of beef carcasses. Meat Sci. 2020;164:108104.
https://doi.org/10.1016/j.meatsci.2020.108104

20. Hugas M, Tsigarida E. Pros and cons of carcass decontamination: The role of the European Food Safety Authority. Meat Sci. 2008;78:43-52.
https://doi.org/10.1016/j.meatsci.2007.09.001

21. Mani-Lŏpez E, García HS, López-Malo A. Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. Food Res Int. 2012;45:713-21.
https://doi.org/10.1016/j.foodres.2011.04.043

22. Van Ba, H, Seo, HW, Pil-Nam S, Kim YS, Park BY, Moon SS, Kang SJ, Choi YM, Kim JH. The effects of pre-and post-slaughter spray application with organic acids on microbial population reductions on beef carcasses. Meat Sci. 2018;137:16-23.
https://doi.org/10.1016/j.meatsci.2017.11.006

23. Nkosi DV, Bekker JL, Hoffman LC. The Use of Organic Acids (Lactic and Acetic) as a Microbial Decontaminant during the Slaughter of Meat Animal Species: A Review. Foods. 2021;10:2293.
https://doi.org/10.3390/foods10102293

24. Zdolec N, Kotsiri A, Houf K, Alvarez-ordóñez A, Blagojevic B, Karabasil N, Salines M, Antic D. Systematic review and meta-analysis of the efficacy of interventions applied during primary processing to reduce microbial contamination on pig carcasses. Foods. 2022b;11: 2110.
https://doi.org/10.3390/foods11142110

25. European Food Safety Authority (EFSA). Evaluation of the safety and efficacy of the organic acids lactic and acetic acids to reduce microbiological surface contamination on pork carcasses and pork cuts. EFSA J. 2018;16:1-76.
https://doi.org/10.2903/j.efsa.2018.5482

26. Commission Regulation (EU) No 101/2013 of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcases.

27. TIBCO Statistica^{TM} Quick Reference, v.13.5. San Ramon, CA, USA: Tibco Software Inc; 2018. Available from: https://docs.tibco.com/products/tibco-data-science-statistica-13-5-0

28. Christiansen P, Krag, R, Aabo, S. Effect of hot water and lactic acid decontamination on *E. coli*, *Salmonella* Typhimurium and *Yersinia enterocolitica* on pork. Book of abstracts of the 8th International Symposium „Safe Pork “; 2009 October 30-1; Quebec, Canada; 2009. pp. 253-7.

29. Morild RK, Olsen JE, Aabo S. Change in attachment of *Salmonella* Typhimurium, *Yersinia enterocolitica*, and *Listeria monocytogenes* to pork skin and muscle after hot water and lactic acid decontamination. Int J Food Microbiol. 2011;145:353-8. https://doi.org/10.1016/j.ijfoodmicro.2010.12.018

30. Grajales-lagunes A, Rivera-bautista C, Ruiz-cabrera M, Gonzales-garcia R, Ramirez-telles J, Abud-archila M. Effect of lactic acid on the meat quality properties and the taste of pork *Serratus ventralis* muscle. Agric Food Sci. 2012;21:171-81. https://doi.org/10.23986/afsci.6082

31. Brustolin JC, Dal pisol A, Steffens J, Toniazzo G, Valduga E, Di luccio M, Cansian RL. Decontamination of pig carcasses using water pressure and lactic acid. Braz Arch Biol Technol. 2014;57:954-61. https://doi.org/10.1590/S1516-8913201402363

32. Dan SD, Mihaiu M, Rotaru O, Dalea I. Microbial changes on the surface of pork carcasses due to lactic and acetic acids decontamination. Bull. 2007;64:403-8. https://doi.org/10.15835/buasvmcn-vm:64:1-2:2450

33. Burin RCK, Silva A, Nero LA. Influence of lactic acid and acetic acid on *Salmonella* spp. growth and expression of acid tolerance-related genes. Food Res Int. 2014;64:726-32. https://doi.org/10.1016/j.foodres.2014.08.019

34. Smulders FJ, Greer GG. Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: prospects and controversies. Int J Food Microbiol. 1998;44:149-69. https://doi.org/10.1016/s0168-1605(98)00123-8

35. Hastaoğlu E, Hastaoğlu, Can ÖP. Lactic acid decontamination in carcass meat. Acad Res J Tech Voc School. 2022;1:36-40.

36. Greer G, Dilts BD. Lactic-acid inhibition of the growth of spoilage bacteria and cold tolerant pathogens on pork. Int J Food Microbiol. 1995;25:141-51. https://doi.org/10.1016/0168-1605(94)00088-n

37. Acuff GR. Chemical decontamination strategies for meat. In: Sofos JN, editor. Improving the Safety of Fresh Meat. Cambridge, UK: Woodhead Publishing; 2005. pp. 350-63. https://doi.org/10.1533/9781845691028.2.350

38. El-tabiy AA, Soliman ZI. Effect of lactic acid and acetic acid on the quality of local meat. Assiut Vet Med J. 2011;57:1-26.
https://doi.org/10.21608/avmj.2011.176680

39. Cosansu S, Ayhan K. Effects of lactic and acetic acid on survival of Salmonella enteritidis during refrigerated and frozen storage of chicken meats. FABT. 2012;5:372-7.
https://doi.org/10.1007/s11947-009-0320-x

40. Yoder SF, Henning WR, Mills EW, Doores S, Ostiguy N, Cutter CN. Investigation of chemical rinses suitable for very small meat plants to reduce pathogens on beef surfaces. J Food Prot. 2012;75:14-21.
https://doi.org/10.4315/0362-028X.JFP-11-084

41. Sallam KJ, Abd-elghany, SM, Hussein MA, Imre K, Morar A, Morshdy AE, Sayed-ahmed MZ. Microbial decontamination of beef carcass surfaces by lactic acid, acetic acid, and trisodium phosphate sprays. BioMed Res Int. 2020;1-11.
https://doi.org/10.1155/2020/2324358

42. Menconi A, Shivaramaiah S, Huff GR, Prado O, Morales JE, Pumford NR, Morgan M, Wolfenden A, Bielke LR, Hargis BM, Tellez G. Effect of different concentrations of acetic, citric, and propionic acid dipping solutions on bacterial contamination of raw chicken skin. Poultry Sci. 2013;92:2216-20.
https://doi.org/10.3382/ps.2013-03172

43. Kassem A, Meade J, Gibbons J, McGill K, Walsh C, Lyng J, Whyte P. Evaluation of chemical immersion treatments to reduce microbial populations in fresh beef. Int J Food Microbiol. 2017;261:19-24.
https://doi.org/10.1016/j.ijfoodmicro.2017.08.005

44. Vaddu S, Kataria J, Rama EN, Moller AE, Gouru A, Singh M, Thippareddi H. Impact of pH on efficacy of peroxy acetic acid against *Salmonella*, *Campylobacter*, and *Escherichia coli* on chicken wings. Poultry Sci. 2021;100:256-262.
https://doi.org/10.1016/j.psj.2020.09.063

45. Choi YM, Kim OY, Kim KH, Kim BC, Rhee MS. Combined effect of organic acids and supercritical carbon dioxide treatments against nonpathogenic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium and *E. coli* O157:H7 in fresh pork. Lett Appl Microbiol. 2009;49:510-5.
https://doi.org/10.1111/j.1472-765X.2009.02702.x

46. Rodriguez-Melcon C, Alonso-Calleja C, Capita R. Lactic acid concentrations that reduce microbial load yet minimally impact colour and sensory characteristics of beef. Meat Sci. 2017;129:169-75.
https://doi.org/10.1016/j.meatsci.2017.01.007

47. Laury AM, Alvarado MV, Nace G, Alvarado CZ, Brooks JC, Echeverry A, Brashears MM. Validation of a lactic acid and citric acid-based antimicrobial product for the reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tips and whole chicken carcasses. J Food Prot. 2009;72:2208-11.
https://doi.org/10.4315/0362-028x-72.10.2208

48. Eggenberger-Solorzano L, Niebuhr SE, Acuff GR, Dickson JS. Hot water and organic acid interventions to control microbiological contamination on hog carcasses during processing. J Food Prot. 2002;65:1248-52.
https://doi.org/10.4315/0362-028x-65.8.1248

49. Tango CN, Mansur AR, Kim GH, Oh DH. Synergetic effect of combined fumaric acid and slightly acidic electrolysed water on the inactivation of foodborne pathogens and extending the shelf life of fresh beef. J Appl.Microbiol. 2014;117:1709-20.
https://doi.org/10.1111/jam.12658

50. Phadtare S, Inouye M. Genome-wide transcriptional analysis of the cold shock response in wild-type and cold-sensitive, quadruple-csp-deletion strains of *Escherichia coli*. J Bacteriol. 2004;186:7007-14.
https://doi.org/10.1128/JB.186.20.7007-7014.2004

51. Cao-Hoang L, Dumont F, Marechal PA, Gervais P. Inactivation of *Escherichia coli* and *Lactobacillus plantarum* in relation to membrane permeabilization due to rapid chilling followed by cold storage. Arch Microbiol. 2010;192:299-305.
https://doi.org/10.1007/s00203-010-0555-y

52. Barria C, Malecki M, Arraiano CM. Bacterial adaptation to cold. Microbiology. 2013;159:2437-43.
https://doi.org/10.1099/mic.0.052209-0

53. Li C, Murugaiyan J, Thomas C, Alter T, Riedel C. Isolate specific cold response of *Yersinia enterocolitica* in transcriptional, proteomic, and membrane physiological changes. Front Microbiol. 2020;10:3037.
https://doi.org/10.3389/fmicb.2019.03037

54. De Boer E, Nouws JFM. Slaughter pigs and pork as source of human pathogenic *Yersinia enterocolitica*. Int J Food Microbiol. 1991;12:375-8.
https://doi.org/10.1016/0168-1605(91)90151-E

55. Kotula AW, Sharar AK. Presence of *Yersinia enterocolitica* O:5/27 in slaughtered pigs. J Food Microbiol. 1993;56:215-8.
https://doi.org/10.4315/0362-028x-56.3.215

56. Dickson JS, Frank JF. Bacterial starvation stress and contamination of beef. Food Microbiol. 1993;10:215-22.
https://doi.org/10.1006/fmic.1993.1023

57. Kocharunchitt C, Mellefont I, Boeman JP, Ross T. Application of chlorine dioxide and peroxyacetic acid during spray chilling as a potential antimicrobial intervention for beef carcasses. Food Microbiol. 2020;87:103355.
https://doi.org/org/10.1016/j.fm.2019.103355

Table 1. List of decontamination protocols used in the experiment

Protocol No.	Description
1.	2 % acetic acid; 25 °C; 10 s
2.	4 % acetic acid; 25 °C; 10 s
3.	2 % acetic acid; 25 °C; 30 s
4.	4 % acetic acid; 25 °C; 30 s
5.	2 % acetic acid; 80 °C; 10 s
6.	4 % acetic acid; 80 °C; 10 s
7.	2 % acetic acid; 80 °C; 30 s
8.	4 % acetic acid; 80 °C; 30 s
9.	2 % lactic acid; 25 °C; 10 s
10.	4 % lactic acid; 25 °C; 10 s
11.	2 % lactic acid; 25 °C; 30 s
12.	4 % lactic acid; 25 °C; 30 s
13.	2 % lactic acid; 80 °C; 10 s
14.	4 % lactic acid; 80 °C; 10 s
15.	2 % lactic acid; 80 °C; 30 s
16.	4 % lactic acid; 80 °C; 30 s
17.	Water; 25 °C; 10 s
18.	Water; 25 °C; 30 s
19.	Water; 80 °C; 10 s
20.	Water; 80 °C; 30 s

Table 2. Comparison of the effectiveness of decontamination protocols based on the used factors (0 h)

	acid	R*	concentration	R	temperature	R	time	R
decontamination protocols/reduction	1	(0.33±0.25)^{a}	1	(0.33±0.25)	1	(0.33±0.25)	1	(0.33±0.25)
	9	(0.70±0.25)^{a}	2	(0.38±0.20)	5	(0.52±0.19)	3	(0.50±0.22)
	2	(0.38±0.20)^{b}	3	(0.50±0.22)	2	(0.38±0.20)	2	(0.38±0.20)
	10	(0.69±0.09)^{b}	4	(0.51±0.23)	6	(0.50±0.18)	4	(0.51±0.23)
	3	(0.50±0.22)^{c}	5	(0.52±0.19)	3	(0.50±0.22)^{a}	5	(0.52±0.19)^{b}
	11	(0.80±0.28)^{c}	6	(0.50±0.18)	7	(0.91±0.38)^{a}	7	(0.91±0.38)^{b}
	4	(0.51±0.23)^{d}	7	(0.91±0.38)	4	(0.51±0.23)^{b}	6	(0.50±0.18)^{c}
	12	(0.86±0.27)^{d}	8	(0.90±0.35)	8	(0.90±0.35)^{b}	8	(0.90±0.35)^{c}
	5	(0.52±0.19)	9	(0.70±0.25)	9	(0.70±0.25)	9	(0.70±0.25)
	13	(0.70±0.26)	10	(0.69±0.09)	13	(0.70±0.26)	11	(0.80±0.28)
	6	(0.50±0.18)^{e}	11	(0.80±0.28)	10	(0.69±0.09)	10	(0.69±0.09)
	14	(0.80±0.27)^{e}	12	(0.86±0.27)	14	(0.80±0.27)	12	(0.86±0.27)
	7	(0.91±0.38)^{f}	13	(0.70±0.26)	11	(0.80±0.28)^{c}	13	(0.70±0.26)^{d}
	15	(1.21±0.18)^{f}	14	(0.80±0.27)	15	(1.21±0.18)^{c}	15	(1.21±0.18)^{d}
	8	(0.90±0.35)	15	(1.21±0.18)	12	(0.86±0.27)^{d}	14	(0.80±0.27)^{e}
	16	(1.24±0.42)	16	(1.24±0.42)	16	(1.24±0.42)^{d}	16	(1.24±0.42)^{e}

* reduction of *Y. enterocolitica* 4/O:3 number ($\log_{10}$ CFU/g)

**values marked with the same letter within the same separate column are statistically different at level 0.05

1. 2% acetic acid; 25 °C; 10 s; **2.** 4% acetic acid; 25 °C; 10 s; **3.** 2% acetic acid; 25 °C; 30 s; **4.** 4% acetic acid; 25 °C; 30 s; **5.** 2% acetic acid; 80 °C; 10 s **6.** 4% acetic acid; 80 °C; 10 s; **7.** 2% acetic acid; 80 °C; 30 s; **8.** 4% acetic acid; 80 °C; 30 s; **9.** 2% lactic acid; 25 °C; 10 s; **10.** 4% lactic acid; 25 °C; 10 s; **11.** 2% lactic acid; 25 °C; 30 s; **12.** 4% lactic acid; 25 °C; 30 s; **13.** 2% lactic acid; 80 °C; 10 s; **14.** 4% lactic acid; 80 °C; 10 s; **15.** 2% lactic acid; 80 °C; 30 s; **16.** 4% lactic acid; 80 °C; 30 s

Table 3. Comparison of the effectiveness of decontamination protocols based on the used factors (24 h)

	acid	R*	concentration	R	temperature	R	time	R
decontamination protocols/reduction	1	(0.71±0.31)	1	(0.71±0.31)	1	(0.71±0.31)	1	(0.71±0.31)
	9	(0.76±0.23)	2	(0.73±0.28)	5	(0.60±0.27)	3	(0.83±0.23)
	2	(0.73±0.28)	3	(0.83±0.23)	2	(0.73±0.28)	2	(0.73±0.28)
	10	(0.57±0.23)	4	(0.82±0.15)	6	(0.70±0.20)	4	(0.82±0.15)
	3	(0.83±0.23)	5	(0.60±0.27)	3	(0.83±0.23)	5	(0.60±0.27)^{e}
	11	(0.85±0.23)	6	(0.70±0.20)	7	(1.04±0.46)	7	(1.04±0.46)^{e}
	4	(0.82±0.15)	7	(1.04±0.46)	4	(0.82±0.15)^{b}	6	(0.70±0.20)^{f}
	12	(0.86±0.31)	8	(1.19±0.56)	8	(1.19±0.56)^{b}	8	(1.19±0.56)^{f}
	5	(0.60±0.27)	9	(0.76±0.23)	9	(0.76±0.23)	9	(0.76±0.23)
	13	(0.79±0.33)	10	(0.57±0.23)	13	(0.79±0.33)	11	(0.85±0.23)
	6	(0.70±0.20)^{a}	11	(0.85±0.23)	10	(0.57±0.23)^{c}	10	(0.57±0.23)^{g}
	14	(1.04±0.33)^{a}	12	(0.86±0.31)	14	(1.04±0.33)^{c}	12	(0.86±0.31)^{g}
	7	(1.04±0.46)	13	(0.79±0.33)	11	(0.85±0.23)^{d}	13	(0.79±0.33)^{h}
	15	(1.15±0.26)	14	(1.04±0.33)	15	(1.15±0.26)^{d}	15	(1.15±0.26)^{h}
	8	(1.19±0.56)	15	(1.15±0.26)	12	(0.86±0.31)	14	(1.04±0.33)
	16	(1.07±0.44)	16	(1.07±0.44)	16	(1.07±0.44)	16	(1.07±0.44)

* reduction of *Y. enterocolitica* 4/O:3 number ($\log_{10}$ CFU/g)

**values marked with the same letter within the same separate column are statistically different at level 0.05

1. 2% acetic acid; 25 °C; 10 s; **2.** 4% acetic acid; 25 °C; 10 s; **3.** 2% acetic acid; 25 °C; 30 s; **4.** 4% acetic acid; 25 °C; 30 s; **5.** 2% acetic acid; 80 °C; 10 s; **6.** 4% acetic acid; 80 °C; 10 s; **7.** 2% acetic acid; 80 °C; 30 s; **8.** 4% acetic acid; 80 °C; 30 s; **9.** 2% lactic acid; 25 °C; 10 s; **10.** 4% lactic acid; 25 °C; 10 s; **11.** 2% lactic acid; 25 °C; 30 s; **12.** 4% lactic acid; 25 °C; 30 s; **13.** 2% lactic acid; 80 °C; 10 s; **14.** 4% lactic acid; 80 °C; 10 s; **15.** 2% lactic acid; 80 °C; 30 s; **16.** 4% lactic acid; 80 °C; 30 s

Table 4. The influence of the main decontamination factors and their interactions on the reduction in the number of *Y. enterocolitica* 4/O:3

Factors		Factor interaction and their significance					
acid	0.096	concentration	0.529	temperature	0.700	time	0.085
		temperature	0.096	time	0.058		
		time	0.171	concentration	0.653		
concentration	0.532	temperature	0.227	time	0.404		
		time	0.796				
temperature	0.001	time	0.044				
time	0.000						

*values less than 0.05 were considered statically significant

Table 5. Comparison of the decontamination effect of acetic acid, lactic acid and water solutions against *Y. enterocolitica* 4/O:3 strains (log10 CFU/g; x±SD)

	reduction	
decontamination protocol	0 h	24 h
2 % acetic acid; 25 °C; 10 s	(0.33±0.25)	(0.71±0.31)
2 % lactic acid; 25 °C; 10 s	(0.70±0.25)^{a}	(0.76±0.23)
4 % acetic acid; 25 °C; 10 s	(0.38±0.20)	(0.73±0.28)
4 % lactic acid; 25 °C; 10 s	(0.69±0.09)^{b}	(0.57±0.23)
water; 25 °C; 10 s	(0.41±0.15)^{ab}	(0.57±0.17)
2 % acetic acid; 25 °C; 30 s	(0.50±0.22)	(0.83±0.23)
2 % lactic acid; 25 °C; 30 s	(0.80±0.28)^{c}	(0.85±0.23)
4 % acetic acid; 25 °C; 30 s	(0.51±0.23)	(0.82±0.15)
4 % lactic acid; 25 °C; 30 s	(0.86±0.27)^{d}	(0.86±0.31)
water; 25 °C; 30 s	(0.56±0.15)^{cd}	(0.80±0.19)
2 % acetic acid; 80 °C; 10 s	(0.52±0.19)	(0.60±0.27)^{h}
2 % lactic acid; 80 °C; 10 s	(0.70±0.26)	(0.79±0.33)
4 % acetic acid; 80 °C; 10 s	(0.50±0.18)	(0.70±0.20)^{i}
4 % lactic acid; 80 °C; 10 s	(0.80±0.27)^{e}	(1.04±0.33)
water; 80 °C; 10 s	(0.54±0.17)^{e}	(0.94±0.32)^{hi}
2 % acetic acid; 80 °C; 30 s	(0.91±0.38)	(1.04±0.46)
2 % lactic acid; 80 °C; 30 s	(1.21±0.18)^{f}	(1.15±0.26)
4 % acetic acid; 80 °C; 30 s	(0.90±0.35)	(1.19±0.56)
4 % lactic acid; 80 °C; 30 s	(1.24±0.42)^{g}	(1.07±0.44)
water; 80 °C; 30 s	(0.81±0.11)^{fg}	(0.98±0.14)

*values marked with the same lowercase letters in the same separate column differ statistically at the 0.05 level

** differences between the individual protocols with acetic acid and lactic acid are shown in the previous tables

Table 6. Average reduction of *Y. enterocolitica* 4/O:3 counts after application of decontamination protocols with organic acids and water ($\log_{10}$ CFU/g; x±SD)

	0 h	24 h
Acetic acid	$(0.56\pm0.21)^{Aa}$	$(0.82\pm0.19)^{a}$
Lactic acid	$(0.87\pm0.22)^{AB}$	(0.88±0.19)
Water	$(0.58\pm0.16)^{Bb}$	$(0.82\pm0.18)^{b}$

*values marked with the same capital letters in the same column are statistically different at the 0.05 level; values marked with the same lowercase letters in the same row are statistically different at the 0.05 level;

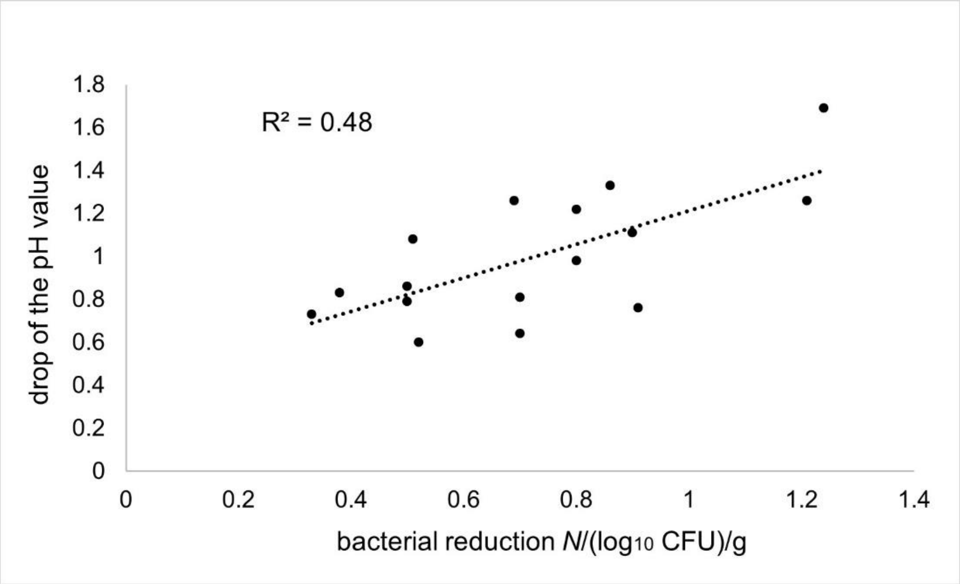

Fig. 1. Correlation between the decrease in the meat pH value and the reduction in the number of *Y. enterocolitica* 4/O:3 (*r*=0.69)

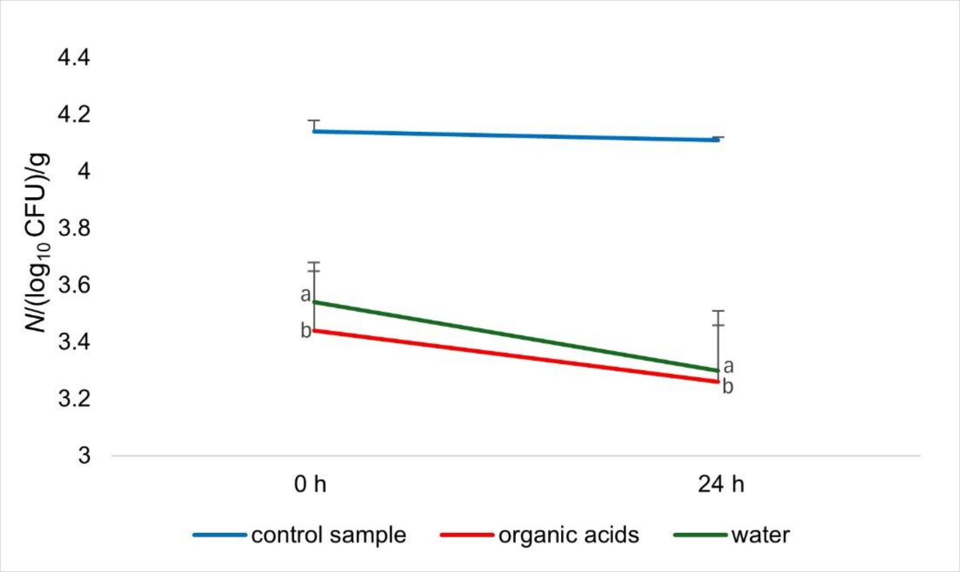

Fig. 2. *Y. enterocolitica* 4/O:3 count after decontamination protocols with organic acid solutions and water at 0 and 24 h (log CFU/g; x±S.D.). Values marked with the same lowercase letters are statistically different at the 0.05 level