



Effect of pH and water activity (a_w) on survival of *Yersinia enterocolitica* in fermented sausages: challenges and risk control

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ARTICLE INFO

Keywords:

Yersinia enterocolitica
Fermented sausages
Water activity
 A_w
PH
Risk control
Microbiological safety

ABSTRACT

Fermented sausages are a popular product in many cultures, but their production can pose a risk due to the presence of pathogenic microorganisms, such as *Yersinia enterocolitica*. This bacterium is known for its ability to survive in meat products, and its survival in fermented sausages is highly dependent on parameters such as pH and water activity (a_w). In this paper, the factors influencing the survival of *Y. enterocolitica* in fermented sausages are investigated, with special emphasis on the control of pH and a_w during production. Also, possible strategies for reducing the risk of contamination in the production process are considered.

1. Introduction

Yersinia enterocolitica is a bacterium that causes zoonotic disease in humans and is associated with consumption of contaminated meat. In fermented sausages, where the meat batter is subjected to the fermentation process, the survival and growth of microorganisms are influenced by various factors, among them pH and water activity (a_w) (Baltić *et al.*, 2011, Baltić *et al.*, 2009). The basic and most important strategy for meat preservation, including of fermented sausages, has been based for centuries on salt and its importance in controlling the growth of spoilage bacteria (especially pathogens), achieving the desired texture and enabling slicing (Montanari *et al.*, 2022). These factors play a key role in

the development or suppression of pathogens (Savić & Savić, 2002). Fermented sausages are considered shelf-stable if their a_w is around 0.92 (<https://www.meatsandsausages.com/sausage-types/fermented-sausage>), and pH is around 6.0 (Oliveres *et al.*, 2010).

2. Materials and methods

For the purposes of the study, category I and II pork meat were used, as well as a certain proportion of pork fat. The meat was ground in a meat grinder to the desired degree of fineness. It was left to cool at a temperature of 0 to 5 °C. After 24 hours, a spice mixture (containing glucose, table salt, spices, and spice extracts) and 2.3% nitrite salt were mixed

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Paper received Jun 2nd 2025. Paper accepted Jun 20th 2025.

The paper was presented at the 63rd International Meat Industry Conference “Food for Thought: Innovations in Food and Nutrition” – Zlatibor, October 05th-08th 2025.

Published by Institute of Meat Hygiene and Technology – Belgrade, Serbia.

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in, and then the minced meat was divided into four groups. A prepared culture of the reference strain *Y. enterocolitica* ATCC 9610 ($8\text{--}9 \log_{10}$ CFU/ml) was added to the stuffing of half the sausages. The starter culture was composed of *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylo-sus*. The starter culture was added in amount of 20 g per 200 kg of ground meat. The prepared stuffing was filled into casings to prepare narrower (34 mm) and wider (55 mm) diameter sausages as follows: the first sausage group (OI) consisted of narrower diameter sausages without a starter culture; the OII group consisted of wider diameter sausages without a starter culture; the OIII group consisted of narrower diameter sausages with a starter culture, and; the OIV group consisted of wider diameter sausages with a starter culture. The sausages were labeled by groups and placed on racks in the smokehouse (smoked at 20–23 °C, dried at 17 °C and a relative air humidity of 75%). The production process lasted 18 days for sausages of narrower diameter and 35 days for sausages of wider diameter.

Microbiological analyses were conducted by counting *Y. enterocolitica* bacteria according to ISO (2017). Chemical and physico-chemical analyses conducted were investigation of pH and a_w . Determination of pH was conducted by the direct method with a pH meter 150 (Testo, Germany) and was performed according to the manufacturer's instructions. The determination of a_w was conducted by using a method described previously (Mitrović et al., 2021). The obtained results were compared through statistical analysis using Microsoft Excel 2010 and GraphPad Prism software, version 8.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All parameters were described by means and standard deviation (SD).

3. Results

At the beginning of the study, the mean number of *Y. enterocolitica* in the stuffing of the experimental (contaminated) groups of narrow and wide diameter sausages was $6.17 \pm 0.04 \log$ CFU/g. In the narrow diameter sausages, i.e., group OI (group to which no starter culture was added), and group OIII (group to which starter culture was added), the average number of *Y. enterocolitica* on day 3 was $4.84 \pm 0.10 \log$ CFU/g and $4.49 \pm 0.04 \log$ CFU/g, respectively, and on day 7 was $5.30 \pm 0.01 \log$ CFU/g and $4.99 \pm 0.04 \log$ CFU/g, respectively. The presence of *Y. enterocolitica* was not detected in sausag-

es with a narrower diameter on days 12 or 18 of the study (end of the production process) or in sausages with a wider diameter on day 25 of ripening.

At the end of ripening (day 18), the mean pH of the OI group sausages (without the added starter culture, narrower diameter) was 5.40 ± 0.06 , and of the OIII group (with added starter culture, narrower diameter) was 5.24 ± 0.08 . On day 35 (also the end of ripening for wider diameter sausages), in the wider sausage diameter OII group (without the addition of starter culture), the pH at the end of ripening was 5.24 ± 0.03 , and in the wider sausage group, OIV (with the addition of starter culture), the mean pH was 5.07 ± 0.05 .

It was found that the average a_w of the sausage stuffing on day 0 of the study was 0.9695 ± 0.0007 . At the end of ripening (day 18), the a_w of the narrower diameter OI group sausages were 0.9200 ± 0.0007 . At the end of ripening, the a_w of the narrower diameter sausages of the OIII group was 0.9235 ± 0.0004 . Also, at the end of ripening (day 35), the a_w of the wider diameter OII group was 0.9359 ± 0.0006 , while the a_w of the wider diameter of the OIV group sausages was 0.9379 ± 0.0005 .

4. Discussion

Food products are often treated with methods to prolong their shelf-life and to contribute to their safety, such as drying that reduces a_w , adding salt or sugar, or pH regulation with natural or added acids (EFSA, 2012). Studies show that using starter cultures, such as *Lactobacillus* spp., can significantly reduce *Y. enterocolitica* levels in fermented sausages. These added bacteria produce lactic acid, bacteriocins, and hydrogen peroxide, which inhibit pathogenic bacteria. Research indicates that proper drying and maturation periods enhance safety.

Lowering pH and reducing a_w through controlled fermentation and drying processes can prevent bacterial survival, growth of pathogens, and also extend the shelf life of the product (Prpich et al., 2021). *Y. enterocolitica* can lead to gastroenteritis, manifesting as diarrhea, abdominal pain, and vomiting. This pathogen is most commonly spread by consuming raw or undercooked meat, unpasteurized milk and contaminated water. *Y. enterocolitica* can survive and even reproduce at low temperatures, meaning refrigerated food can still be a source of infection. In rare cases, the bacterium can lead to severe complications such as septicemia, arthritis, or erythema nodosum (Ivanović et al., 2016).

Y. enterocolitica is known for its ability to survive in a wide range of pH values, but an acidic environment significantly reduces its viability (Leroy *et al.*, 2015). The combination of low a_w and acidic pH can be an effective strategy to control this bacterium in food products (Barbieri *et al.*, 2021).

Y. enterocolitica and other microorganisms require a certain availability of water for growth and survival. However, reducing the a_w can limit the growth of bacteria, molds and yeasts (Mitrovic, 2016). For example, bacteria usually cannot survive when the a_w is below 0.9, while molds stop growing below a_w 0.7 (Laranjo *et al.*, 2019).

5. Conclusion

During the ripening of the fermented sausages, there was a decrease in the a_w and number of *Y.*

enterocolitica. The number of *Y. enterocolitica* bacteria was lower on all test days in the sausages to which the starter culture was added. The presence of *Y. enterocolitica* was not detected in the sausages with a narrower diameter on day 12, or in sausages with a wider diameter on day 25 of ripening.

The pH of all examined groups of fermented sausages decreased during the ripening process, and at the end of ripening it was lower in sausages with a wider diameter and in sausages in which starter cultures were used.

During the ripening process, the a_w of the control and experimental groups of fermented sausages of narrower and wider diameter decreased, but was higher in the sausages in which starter cultures were used.

Disclosure Statement: No potential conflict of interest was reported by the authors.

Funding: The research results presented in this paper were funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, based on the Agreement on the Implementation and Funding of Scientific Research Work of the Scientific Research Organization (SRO) in 2025, No. 451-03-136/2025-03/200050 dated February 4, 2025.

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