Kırklareli Üniversitesi
Mühendislik ve Fen Bilimleri Dergisi
Cilt 11, Sayı 1, 1-10, 2025

Kırklareli University
Journal of Engineering and Science
Volume 11, Issue 1, 1-10, 2025

Research Article DOI: 10.34186/klujes.1562932

Effect of Hydrochloric Acid Application on the Detection of *Salmonella* spp. and *Escherichia coli* O157:H7 in Food Samples

Sevda Terzi Yavaş[1], Deniz Yüksel Yence[1], Ece Şen[1*]

[1]*Department of Biology, Faculty of Science, Trakya University, Edirne, Türkiye*

Received: 07.10.2024, Accepted: 25.12.2024, Published: 07.02.2025

ABSTRACT

In this study, it was investigated whether treatment of food samples with HCl (prepared with NaCl solution) could be used instead of the pre-enrichment and enrichment steps in the ISO methods used for the detection of *Salmonella* spp. and *E. coli* O157:H7 in food samples. For this purpose, 103 food samples were analysed for *Salmonella* spp. and *E. coli* O157:H7 detection with 3 different methods, including ISO and 2 modified methods. In the modified methods, food samples were treated with five different concentrations of HCl instead of or after the enrichment step. *E. coli* O157:H7 (beef meat and Turkish-style fermented sausage) and *Salmonella* spp. (whipped cream and instant soup powder) were detected in 2 of the food samples examined. The same results were obtained for the detection of *E. coli* O157:H7 and *Salmonella* spp. in food samples with 3 different methods used. However, effective HCl concentrations (ranging from 1/2 N to 1/10 N) varied depending on the pathogen detected and food type. The results showed that the treatment of food samples with 1/10 N HCl instead of the enrichment step was effective in both the detection of *Salmonella* spp. and *E. coli* O157:H7 in different food samples.

Keywords: Foodborne pathogen; *Salmonella* spp.; *Escherichia coli* O157:H7; detection; hydrochloric acid.

Gıda Örneklerinde *Salmonella* spp. ve *Escherichia coli* O157:H7'nin Tespitininde Hidroklorik Asit Uygulamasının Etkisi

ÖZ

Bu çalışmada, gıda numunelerinin HCl ile muamele edilmesinin (NaCl çözeltisi ile hazırlanmış) gıda numunelerinde *Salmonella* spp. ve *E. coli* O157:H7 tayini için kullanılan ISO yöntemlerindeki ön zenginleştirme ve zenginleştirme adımları yerine kullanılıp kullanılamayacağı araştırılmıştır. Bu amaçla, toplam 103 gıda örneği ISO metod ve 2 modifiye edilmiş yöntem olmak üzere 3 farklı yöntem kullanılarak *Salmonella* spp. ve *E. coli* O157:H7 tespiti için analiz edilmiştir. Modifiye edilmiş yöntemlerde, gıda örnekleri zenginleştirme adımı yerine veya sonrasında beş farklı HCl konsantrasyonu ile muamele edilmiştir. İncelenen gıda örneklerinin 2'sinde *E. coli* O157:H7 (dana eti ve Türk usulü fermente sucuk) ve *Salmonella* spp. (çırpılmış krema ve hazır çorba tozu) tespit edilmiştir. Gıda örneklerinde *E. coli* O157:H7 ve *Salmonella* spp. tespiti için kullanılan 3 farklı yöntemle aynı sonuçlar elde edilmiştir. Ancak etkili HCl konsantrasyonları (1/2 N ila 1/10 N aralığında) tespit edilen patojene ve gıda türüne bağlı olarak değişmiştir. Elde edilen sonuçlar, gıda örneklerinin zenginleştirme adımı yerine 1/10 N HCl ile muamele edilmesinin farklı gıda örneklerinde hem *Salmonella* spp. hem de *E. coli* O157:H7 tespitinde etkili olabileceğini göstermiştir.

Anahtar Kelimeler: Gıda kaynaklı patojen; *Salmonella* spp.; *Escherichia coli* O157:H7; tespit; hidroklorik asit

**Corrseponding Author: Ece ŞEN*
ecesen@trakya.edu.tr

1. INTRODUCTION

Foodborne pathogens are a major concern all over the world. *Escherichia coli* O157:H7 and *Salmonella* spp. are among these pathogens that pose public health and economic importance. These microorganisms can cause serious illnesses and even death, especially for pregnant women, infants, children, elderly people, and people with compromised immune systems (Panisello et al., 2000; Rangel et al., 2005). Foodborne outbreaks are related to the consumption of contaminated foods. For *E. coli* O157:H7, foods including hamburger meat, unchlorinated water, bruised apples, milk, unpasteurized apple juice, potatoes, lettuce, and mayonnaise are considered the major sources (Deisingh & Thompson, 2004). Food sources for *Salmonella* spp. can differ for different serovars. Although raw eggs, pork, beef, and poultry are the primary sources of contamination for non-typhoidal serovars, seafood, fruits, and vegetables are also sources of contamination (Ferrari et al., 2019). For this reason, early detection of pathogens and the source of pathogens in foodborne diseases has an important place in terms of public health and preventing the spread of diseases.

For the separation and detection of a specific microbial species, plating a mixed culture directly onto selective media can often result in low efficiency, as the target microorganism may be present in low numbers and may not grow well in the selective media. This is particularly true for complex samples, such as foods that can contain a mixed background flora (Safarik et al., 1995). Selective enrichment is commonly used to recover *E. coli* O157:H7 from a mixed culture (Chapman, 2000). The immunomagnetic separation (IMS) technique is a simple and effective method to isolate and concentrate *E. coli* O157:H7 from enriched samples (Bai et al., 2022).

Traditional culture methods for the detection and isolation of *Salmonella* spp. are still widely used as the gold standard strategy. This method involves the use of selective and differential media that are designed to isolate and identify *Salmonella* spp. based on their unique characteristics. The ISO method in use includes pre-enrichment and selective enrichment steps for the detection of pathogens in food samples (ISO 6579-1:2017). One of the major drawbacks of the standard microbial culture method used for *Salmonella* spp. detection is the long waiting time involved. This method can take several days to produce results, which can be a significant drawback when timely detection and treatment of infections is critical (Wang et al., 2021).

In this study, it was investigated whether the detection of *Salmonella* spp. and *E. coli* O157:H7 in food samples could be done more quickly and economically by using cultural methods. For this purpose, the standard ISO method and 2 modified versions were tested in various food samples. In the modified methods, food samples were treated with different HCl dilutions prepared with NaCl instead of or after the enrichment step.

2. MATERIAL AND METHOD

2.1. Sample collection

103 food samples included meat samples (89), instant soup powders (3), whipped creams (2), Turkish-style fermented sausages (2), raw milk (1), yogurt (1), ketchup (1), lettuce (1), nut (1), fruit juice (1) and mayonnaise (1) were analyzed. Samples were stored under defined food storage conditions during transportation and analysis (Table 1). *E. coli* O157:H7 (ATCC 43888) and *Salmonella enterica* serovar Enteritidis ATCC 13076 strains were used for the determination of the limits of detection assays.

Table 1: Sample storage conditions and pH values

Food sample	**pH value**	**Temperature (°C)**
Meat samples	Between 5,37 - 5,74	4,2
Turkish-style fermented sausages	5,46 and 5,48	4,4
Whipped creams	6,08 and 6,05	4,5
Instant soup powders	4,42	4,5
Yogurt	4,42	4,6
Raw milk	6,08 and 6,05	4,4
Lettuce	6,47	4,3
Nut	5,46 and 5,48	4,2
Mayonnaise	4,02	3,9
Ketchup	3,75	4,1

Table 2: HCl dilutions and pH values

HCl dilutions (N)	**pH values**
1/100	1,95
1/40	1,58
1/35	1,51
1/30	1,47
1/25	1,38
1/10	1,01
1/8	1,00
1/6	0,77
1/4	0,74
1/2	0,50

2.2. Preparing HCl dilutions for acid treatment of food samples

1N HCl solution was prepared with distilled water and 0,5% NaCl solution was used to prepare HCl dilutions. HCl dilutions used in the assay and the pH values of the solutions are shown in Table 2.

2.3. Standart Detection Procedure for E. coli O157:H7

The detection of *E. coli* O157:H7 was performed using ISO 16654:2001/Amd 1:2017. 25 g (or 25 mL) food sample was aseptically taken and added to 225 mL of Tryptic-Soy-Broth with Novobiocin (Merck, Germany), homogenized for 2 min, and incubated for 6 - 18 h at 41,5°C for enrichment. After incubation time, one mL of culture was added to microcentrifuge tubes containing 20 µL of IMS particles. Microcentrifuge tubes were incubated in a shaker at 20 rpm for 30 min. A magnetic separator was used to collect the beads and supernatant was removed. IMS particles were washed twice with 1 mL wash buffer (Modified Phosphate Buffer). After that, the wash buffer was removed and 100 µL of wash buffer was added to each microcentrifuge tube and they were mixed by vortexing for 30 sec and one min. 50 µL of these cultures were streaked onto Sorbitol MacConkey Agar with Cefixime & Tellurite (CT-SMAC) and incubated for 24±2 h at 37°C. Transparent and almost colorless, yellowish-brown pale-looking colonies were determined as *E.coli* O157:H7.

2.4. Detection procedure with HCl treatment without enrichment for E. coli O157:H7

One g (or one mL) of food samples was aseptically taken and added to different HCl dilutions (1/10N, 1/8N, 1/6N, 1/4N, and 1/2N) and they were mixed by vortexing for 30 sec and one min. After incubation time, the immunomagnetic separation process was performed as described above. 50 µL of these cultures was streak onto Sorbitol MacConkey Agar with Cefixime & Tellurite (CT-SMAC) agar and incubated for 24±2 h at 37°C. Transparent and almost colorless, yellowish-brown pale-looking colonies were determined as *E. coli* O157:H7.

2.5. Detection procedure with HCl treatment after enrichment for E. coli O157:H7

25 g (or 25 mL) food samples were aseptically taken and added to 225 mL of Tryptic-Soy-Broth with Novobiocin (Merck, Germany), homogenized for 2 min, and incubated for 6 - 18 h at 41.5°C for enrichment. After incubation time, one mL of these cultures was added to different HCl dilutions (1/10, 1/8, 1/6, 1/4, and 1/2N) and mixed by vortexing for 30 sec and 1 min. The

immunomagnetic separation process was performed as described above. 50 µL of these cultures was streaked on Sorbitol MacConkey Agar with Cefixime & Tellurite (CT-SMAC) and incubated for 24±2 h at 37°C. Transparent and almost colorless, yellowish-brown pale-looking colonies were determined as *E. coli* O157:H7.

2.6. Standard detection procedure for Salmonella spp.

The detection of *Salmonella* spp. was performed using the ISO 6579-1:2017 method. 25 g (or 25 mL) food samples were aseptically taken and added into 225 mL of Buffered Peptone Water (BPW) (Merck, Germany) homogenized for two min and incubated for 18 h at 37°C for pre-enrichment. One mL of these cultures was added into 10 mL of Mueller Kauffmann Tetrathionate Novobiocin Broth (MKTTn) (LABM, England) for 24±2 h at 37°C and also 0.1 mL of these cultures were added to RAPPAPORT-VASSILIADIS-Soya broth (RVS) (Merck, Germany) for 24 ± 2 h at 41.5°C for enrichment. A loopful of both MKTTn broth and RVS broth culture was streaked onto XLD and BGA agar and incubated for 24 ± 2 h at 37°C. Red colonies with a black zone on XLD agar and red or pinkish-white colonies surrounded by a red halo on BGA agar were determined as *Salmonella* spp.

2.7. Detection procedure with HCl treatment without enrichment for Salmonella spp.

One g (or one mL) food sample was aseptically taken and added into different HCl dilutions (1/10, 1/8, 1/6, 1/4, and 1/2N) and incubated for 30 sec and one min at room temperature. A loopful of this solution was streaked onto XLD and BGA agar and incubated for 24 ± 2 h at 37°C. Red colonies with black zone on XLD agar and pinkish-white or red colonies surrounded by a red halo on BGA agar were determined as *Salmonella* spp.

2.8. Detection procedure HCl treatment after enrichment for Salmonella spp.

25 g (or 25 mL) of each food sample was aseptically taken and added in 225 mL of Buffered Peptone Water (Merck, Germany) homogenized for 2 min and incubated for 18 h at 37°C for pre-enrichment. After incubation time, one mL of these cultures was added to different HCl dilutions (1/10, 1/8, 1/6, 1/4, and 1/2N) and they were mixed by vortexing for 30 sec and one min, a loopful of these solutions was streaked onto XLD and BGA agar and incubated for 24 ± 2 h at 37°C. Red colonies with a black zone on XLD agar and red or pinkish-white colonies surrounded by a red halo on BGA agar were determined as *Salmonella* spp.

2.9. Determination of the effect of HCl treatment on E. coli O157:H7 and Salmonella spp.

Overnight cultures of *E. coli* O157:H7(ATCC 43888) and *S.* Enteritidis ATCC 13076 strains were diluted with BPW to 1 McFarland standard ($3x10^9$ cfu/ml). Dilutions were prepared to contain $3x10^8$ cfu/mL of bacteria. One mL of these dilutions was added to 1/2, 1/4, 1/6, 1/8, 1/10, 1/25, 1/30, 1/35, 1/40, and 1/100 N HCl solutions and mixed by vortexing for 30 sec and one min. A loopful of these solutions was streaked onto CT-SMAC agar (for *E. coli* O157:H7) and also XLD and BGA agar (for *Salmonella* spp.) and incubated for 24 ± 2 h at 37°C. Colony counts were compared to determine the effectiveness of treatment procedures.

3. RESULTS AND DISCUSSION

The detection and isolation of foodborne pathogens such as *E. coli* O157: H7 and *Salmonella* spp. have a great importance for public health. However, a high background microbiota poses an obstacle to the detection and isolation of these pathogens. This can make it difficult to differentiate between the target pathogen and other microorganisms that are present in the sample. For foodborne pathogens to cause disease in humans, they must first survive in the acidic condition of the stomach. *Salmonella* spp. and *E. coli* are known to have more tolerance to acid than other Gram-negative bacteria (Gorden & Small, 1993). HCl treatment has been used to separate these microorganisms from background microbiota in feces and food. HCl treatment is a low-cost, easy-to-apply, and fast procedure. Our study investigated the effectiveness of HCl treatment in the detection of *Salmonella* spp. and *E. coli* O157:H7 from various food samples. For this purpose, ground meat samples (n=89) and ten different food samples (n=14) were used. The ISO method and also two modified methods were used for the detection of *Salmonella* spp. and *E. coli* O157:H7. Food samples were treated with five different concentrations of HCl (1/10, 1/8, 1/6, 1/4, and 1/2N) instead of or after the enrichment step.

E. coli O157: H7 was detected in 2 (1,9 %) of 103 food samples using both classical and HCl treatment procedures. These samples were one beef meat and one Turkish-style fermented sausage. The same results were obtained from both the classical and also HCl treatment procedures. Treatment of food samples in 1/2 and 1/10 N and only 1/10 N HCl concentrations was effective for beef meat and Turkish-style fermented sausage sample, respectively. The time of the HCl treatment (30 sec or one min) did not change the results (Table 3). In these two food samples (beef meat and also Turkish-style fermented sausage), the same HCl concentrations were effective for

treatment both after pre-enrichment and without pre-enrichment. For two different food samples, 1/10 N HCl treatment (for 30 sec and 1 min) was effective. Several studies have been conducted using HCl treatment in the detection of *E. coli* in meat, sprouts, and faeces to improve the effectiveness of the detection method. Fukushima and Gomyada (1999) found that 1/8N HCl treatment (for 30 sec) increased the sensitivity for detection of *E. coli* 0157:H7 from fecal samples and enrichment cultures of a variety of samples. Fedio et al. (2012) showed that the use of a short acid treatment after enrichment and post-enrichment using IMS beads improved *E. coli* O157:H7 isolation from alfalfa sprouts. Also, Lamparter et al. (2020) showed that an acid treatment added to the enrichment step during the isolation of Shiga toxin-producing *E. coli* from sprouts increased the success of isolation. In all of these studies, HCl treatment was used with an enrichment step. Our study revealed that HCl treatment (1/10 N) can be used instead of the enrichment step for *E. coli* O157:H7 detection from different food samples.

Salmonella spp. was detected in 2 (1,9 %) of 103 food samples by using both classical and HCl treatment procedures. These samples were one instant soup powder and one whipped cream. The same results were obtained from both classical and also HCl treatment procedures (without and after the enrichment step). Treatment of food samples in 1/10 and 1/6 N HCl concentrations was effective for the whipped cream sample, and also for the treatment of food samples in 1/10, 1/8 and 1/6 N HCl concentrations was effective for the instant soup powder sample. The time of the HCl treatment (30 sec or 1 min) did not change the results (Table 3). Barkocy-Gallagher et al. (2002), in their study to develop effective methods for *E. coli* O157:H7 and *Salmonella* spp. isolation from fecal samples, hide, and bovine carcass, and, also tested whether mild acid shock would be effective in non-selective pre-enrichment. However, they found that the non-selective pre-enrichment step (without acid) was more effective. The results obtained from our study showed that HCl treatment can be used instead of enrichment steps in both food samples in which *Salmonella* spp. was detected. However, the effective concentrations in different food samples also varied. However, it seems that 1/10 N and 1/6 N HCl concentrations can be used for both samples.

The effect of HCl treatment of *E. coli* O157:H7 ATCC 48333 and *S.* Enteritidis ATCC 13076 was determined. According to these results, for *E. coli* O157:H7 ATCC 48333, 0.5% NaCl solution did not affect bacterial growth, while HCl solutions prepared at 10 different concentrations used in the study inhibited the growth. For *S.* Enteritidis ATCC 13076, it was determined that both 0.5% NaCl solution and HCl solutions prepared at 10 different concentrations inhibited bacterial growth. The

obtained results of our assays showed that HCl treatment inhibits bacterial growth. It is thought that these results are due to the fact that the standard strains used in the experiments were kept in the culture collections and have been passaged for many years which might have affected the physiological features and growth behavior of these bacteria.

Table 3: *Salmonella* spp. and *E. coli* O57:H157 detection in various food samples

	***E. coli* O57:H157**			***Salmonella* spp.**		
Food samples (count)	**HCl treatment without enrichment**	**HCl treatment after enrichment**	**Standard procedure**	**HCl treatment without enrichment**	**HCl treatment after enrichment**	**Standard procedure**
Meat samples (88)	N	N	N	N	N	N
Meat sample (1)	1/2 N HCl P	1/2, 1/10 HCl P	P	N	N	N
Instant soup powders (2)	N	N	N	N	N	N
Instant soup powder (1)	N	N	N	1/10 N HCl P	1/6, 1/8, 1/10N HCl P	P
Turkish-style fermented sausage (1)	N	N	N	N	N	N
Turkish-style fermented sausage (1)	1/10 N HCl P	1/10 N HCl P	P	N	N	N
Whipped cream (1)	N	N	N	N	N	N
Whipped cream (1)	N	N	N	1/6, 1/10 N HCl P	1/6, 1/10 N HCl P	P
Yogurt (1)	N	N	N	N	N	N
Lettuce (1)	N	N	N	N	N	N
Mayonnaise (1)	N	N	N	N	N	N
Fruit juice (1)	N	N	N	N	N	N
Ketchup (1)	N	N	N	N	N	N
Nut (1)	N	N	N	N	N	N
Raw milk (1)	N	N	N	N	N	N

N: negative, P: positive

4. CONCLUSION

In conclusion, for *E. coli* 0157:H7 and *Salmonella* spp. detection from food samples based on cultural methods included pre-enrichment and enrichment steps. These steps involve incubating the food samples in specific growth media to increase the counts of bacteria in the sample, making it easier to detect the pathogens of interest. However, these steps can take several hours or even days to complete. Our results show that subjecting food samples to HCl treatment in the *E. coli*

0157:H7 and *Salmonella* spp. detection procedure in different food samples eliminates the need for pre-enrichment and enrichment steps. This both shortens the time required to detect these pathogens and reduces the cost.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest among the authors.

ACKNOWLEDGMENTS

This article was produced from the first author's (Sevda Terzi Yavaş) master's thesis under the supervision of Dr. Ece Şen. This research was funded by the Trakya University Scientific Research Fund by the research project, grant number 2018/133.

AUTHOR CONTRIBUTIONS

S.T.Y. : Methodology, resources, writing—original draft preparation.

D.Y.Y. : Formal analysis, writing—original draft preparation, review and editing.

E.Ş. : Validation, formal analysis, writing—original draft preparation, review and editing, supervision, project administration, funding acquisition.

REFERENCES

Bai, Z., Xu, X., Wang, C., Wang, T., Sun, C., Liu, S., & Li, D. (2022). A comprehensive review of detection methods for *Escherichia coli* O157: H7. *TrAC Trends in Analytical Chemistry*, 152, 116646.

Barkocy-Gallagher, G. A., Berry, E. D., Rivera-Betancourt, M., Arthur, T. M., Nou, X., & Koohmaraie, M. (2002). Development of methods for the recovery of *Escherichia coli O157: H7* and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *Journal of Food Protection*, 65(10), 1527-1534.

Chapman, P. A. (2000). Methods available for the detection of *Escherichia coli* O157 in clinical, food and environmental samples. *World Journal of Microbiology and Biotechnology*, 6, 733–740.

Deisingh, A. K., & Thompson, M. (2004). Strategies for the detection of *Escherichia coli* O157: H7 in foods. *Journal of Applied Microbiology*, 96(3), 419-429.

Fedio, W. M., Jinneman, K. C., Yoshitomi, K. J., Zapata, R., & Weagant, S. D. (2012). Efficacy of a post enrichment acid treatment for isolation of *Escherichia coli* O157: H7 from alfalfa sprouts. *Food Microbiology*, 30(1), 83-90.

Ferrari, R. G., Rosario, D. K., Cunha-Neto, A., Mano, S. B., Figueiredo, E. E., & Conte-Junior, C. A. (2019). Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. *Applied and Environmental Microbiology*, 85(14), e00591-19.

Fukushima, H., & Gomyoda, M. (1999). An effective, rapid and simple method for isolation of Shiga toxin-producing *Escherichia coli* 026, 0111 and 0157 from faeces and food samples. *Zentralblatt für Bakteriologie*, 289(4), 415-428.

Gorden, J., & Small, P. (1993). Acid resistance in enteric bacteria. *Infection and immunity*, 61(1), 364-367.

ISO 16654:2001/Amd 1:2017. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157 — Amendment 1: Annex B: Result of interlaboratory studies.

ISO 6579-1:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp.

Lamparter, M. C., Seemann, A., Hobe, C., & Schuh, E. (2020). Using hydrochloric acid and bile resistance for optimized detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC) from sprouts. *International Journal of Food Microbiology*, 322, 108562.

Panisello, P. J., Rooney, R., Quantick, P. C., & Stanwell-Smith, R. (2000). Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. *International Journal of Food Microbiology*, 59(3), 221-234.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157: H7 outbreaks, united states, 1982–2002. *Emerging Infectious Diseases*, 11(4), 603.

Safarik, I., Safarikova, M., & Forsythe, S. J. (1995). The application of magnetic separations in applied microbiology. *Journal of Applied Microbiology*, 78(6), 575-585.

Wang, M., Zhang, Y., Tian, F., Liu, X., Du, S., & Ren, G. (2021). Overview of rapid detection methods for *Salmonella* in foods: Progress and challenges. *Foods*, 10(10), 2402.