

Retail Risk Assessment and Lethality of *Listeria monocytogenes* and *E. coli* O157 in Naturally Fermented Sauerkraut

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Abstract The interest in fermenting foods at retail and food service levels is increasing. Foodborne pathogens such as *E. coli* O157:H7 and *Listeria monocytogenes*, however, have been implicated in foodborne illness in several fermented and acidic foods. This study evaluated and validated the lethality of potentially acid-tolerant pathogens *E. coli* O157 and *L. monocytogenes* in sauerkraut that was made using traditional fermentation techniques. Fresh cabbage juice prepared with 2.5% salt was inoculated separately with a 5-strain mixture of *E. coli* O157 and a 5-strain mixture of *L. monocytogenes* and then was allowed to ferment at 25 °C. The pH decreased at a steady rate for the first 7 days and remained relatively stable thereafter. There was a significant decrease in *E. coli* O157 from Day 1 to Day 7 ($p < .05$) and a significant decrease in *L. monocytogenes* count from Day 2 to Day 7 ($p < .05$) with a 5-log reduction for both pathogens at Day 7 and no pathogens detected after Day 9. The data indicate that fermentation of cabbage at ambient temperature is lethal to the survival of *E. coli* O157 and *L. monocytogenes*. This study can be used to support the safety of sauerkraut fermentations in retail and food service operations.

Introduction

Recent interest in traditional foods and probiotics has brought fermented sauerkraut back in vogue among both consumers and retail food service operators. As a fermented food, sauerkraut is designated as a special process under the Food and Drug Administration model *Food Code* (U.S. Department of Health and Human Services [HHS], 2017). Specifically, fermentation is considered a special process where a food additive (e.g., bacterial culture) is used to make a potentially hazardous (i.e., time/temperature control for safety [TCS]) food into a nonpotentially hazardous (non-TCS) food (HHS, 2017). As a *Food Code* special process, fermentation requires a

documented and implemented hazard analysis critical control point (HACCP) food safety plan (HHS, 2017).

Intrinsic and Extrinsic Properties of Sauerkraut

The main ingredient in sauerkraut is cabbage, typically with an addition of 2–3% salt (Pérez-Díaz et al., 2013). More specifically, Bavarian- or German-style sauerkraut might use red or green head cabbage, onions, and caraway seeds. Chinese-style sauerkraut might be fermented from Chinese cabbage only, also known as baechu (Park, 2017). It has been noted that there are likely as many different sauerkraut recipes as there are sauerkraut makers.

The traditional sauerkraut fermentation comes from a natural proliferation of resident lactic acid bacteria (LAB). Numerous studies have identified a two-stage anaerobic lactic acid fermentation. Heterofermentative LAB such as *Leuconostoc mesenteroides* start growing quickly (within 24–48 hr), leading to the production of lactic acid primarily, with some acetic acid, alcohol, and carbon dioxide (CO₂) also produced (Steinkraus, 2002). The CO₂ bubbles are an effective sign that heterolactic fermentation is active. As more acid develops, the acid-tolerant homofermentative LAB, such as *Lactobacillus plantarum*, overtake the heterolactic fermenters to grow and produce additional lactic acid. *L. plantarum* usually will grow until all of the degradable carbohydrates are used up or when *L. plantarum* reaches a pH level where it can no longer grow (Steinkraus, 2002). The pH of cabbage is approximately 6–6.5 and the pH of most sauerkraut is between 3.4 and 3.7 (Plengvidhya et al., 2007), but can go as low as 3.2 with a final lactic acid concentration between 1.5 and 2.5% (Pérez-Díaz et al., 2013).

Sauerkraut Hazards

Foodborne pathogens of concern are present in the farm environment and can contaminate the raw cabbage via irrigation water, unhygienic human handling, and fertilizers that are made from animal feces (Niksic et al., 2005). Further unhygienic handling, cross-contamination, and improper processing can introduce and allow proliferation of pathogens in the retail food service or artisanal processing environment. Bacterial hazards from fresh vegetables include most of the common vegetative and spore-forming pathogens, except those of seafood origin (e.g., *Vibrio*). Salt and rapid acidifi-

cation historically have been believed to account for the inhibition of spore-forming pathogens (Breidt & Fleming, 1998). Modeling *Clostridium botulinum* growth using a ComBase predictive model (www.combase.cc) demonstrates a lag time of 15–24 hr at 25 °C with a 5.5–6.0 pH and a 2% salt concentration. These conditions allow the faster-growing lactic acid fermentation culture to grow and produce lactic acid, which further increases the inhibition of spore-forming pathogens.

After sauerkraut fermentation is complete, commercial producers usually employ in-container pasteurization to destroy any potential vegetative pathogens and most organisms that contribute to food spoilage. Many retail food service operators and artisanal producers, however, do not pasteurize their final product. Pasteurization reduces the crisp texture of the cabbage and will kill off the desired live LAB culture. If the sauerkraut is not pasteurized, acid-tolerant vegetative pathogens (e.g., *E. coli* O157:H7 and *Listeria monocytogenes*) can remain. Various acidic foods such as apple cider, mayonnaise, yogurt (Hsin-Yi & Chou, 2001), and kimchi (Shin et al., 2016) have been implicated in the outbreaks of foodborne disease caused by *E. coli* O157:H7 or related Shiga toxin-producing *E. coli* strains. Additionally, *L. monocytogenes* has been associated with outbreaks in some acidic foods including fermented sausages and has shown the ability to survive in some acidic foods (Gandhi & Chikindas, 2007). *L. monocytogenes* has been found to survive in both the fermentation stage at room temperature as well as in the refrigeration stage in home-fermented refrigerator dill pickles for up to 91 days (Kim et al., 2005).

Sauerkraut Controls

There are three generally accepted bacterial pathogenic hazard control factors in sauerkraut fermentations: 1) salt, 2) competitive LAB cultures, and 3) the rapid production of lactic acid and other acids. The traditional sauerkraut fermentation process, like most vegetable fermentations, requires salt (Pérez-Díaz et al., 2013). The role of salt is to slow the growth of food spoilage microorganisms, allowing LAB to proliferate (Taormina, 2010). The second control factor is the presence of a competitive fermentation culture.

Previous work in our laboratory on the survival of pathogens in low-salt cheddar cheese suggests that the fermentation cultures and their by-products contributed heavily toward the control of pathogen growth (Shrestha et al., 2011a, 2011b).

The last control factor is perhaps the most significant. The production of fermentation acids is rapid in most cases of lactic acid fermentation of sauerkraut (Beganovi et al., 2014; Plengvidhya et al., 2007). Lactic acid has shown to be highly inhibitory as both an organic acid and via its ionic effect on pH. As an organic acid, the protonated form enters the bacterial cell more freely to disassociate inside the cell, leading to toxicity (Breidt, 2005). It is ultimately the intracellular pH value that affects bacterial growth and survival. Acid-tolerant pathogenic bacteria have developed mechanisms to resist the intracellular pH change (Cotter & Hill, 2003). They usually succumb, however, under conditions of active metabolism when the concentration of organic acid reaches a critical point. The fermentation LAB generally are acid tolerant. Specifically, *L. mesenteroides* generally stops growth at a pH level of 4.0 and *L. plantarum* grows and survives at pH levels ≤ 3.5 (Breidt, 2005; McDonald et al., 1990).

This study was performed to support that the hazard controls of salt, LAB growth, and acid production in traditional sauerkraut fermentations preclude growth of all foodborne bacterial pathogens and demonstrate that the potentially acid-tolerant pathogens *E. coli* O157 and *L. monocytogenes* do not survive.

Methods

Sauerkraut Preparation

We obtained fresh green head cabbage from a local grocery store. We chopped the cabbage (unrinsed) and extracted the juice using a vegetable juicer. We added noniodized salt at 2.5% to the juice. The juice was used immediately and kept at room temperature (25 °C) for fermentation.

Inoculum Preparation

For *L. monocytogenes*, we obtained five strains (FSL J1-177, FSL C1-056, FSL N3-013, FSL R2-499, and FLS N1-227) from the culture collection of Dr. Jeff Broadbent at Utah State University. Similarly for *E. coli* O157, we obtained five strains of vegetable origins

or related to vegetable outbreaks (H1730, EC4042, EC4045, EC4191, and EC4206) from the culture collection of Dr. Donald Schaffner at Rutgers University. Pure cultures were maintained as frozen stocks at -80 °C.

Cultures for each strain were prepared by transferring 0.1 ml of thawed frozen stock into 10 ml of fresh tryptic soy broth (TSB) and incubating at 37 °C for 24 hr. For *L. monocytogenes*, strains were plated into PALCAM agar and incubated at 37 °C for 48 hr. For *E. coli* O157, individual strains were plated into MacConkey Sorbitol agar and incubated at 37 °C for 24 hr. A working culture for each strain was then grown in TSB at 37 °C for 24 hr. The 5-strain mixture for each pathogen was prepared by combining 2-ml aliquots of each strain in a 15-ml conical centrifuge tube. Cells were pelleted by centrifugation (1,509 \times g for 15 min) and resuspended in 10 ml of Butterfield PBS solution 3 times. Appropriate dilutions of washed cell suspensions were prepared in Butterfield PBS to achieve approximately 107 cells/g of sample.

Sample Inoculation and Incubation

We distributed the freshly prepared cabbage juice equally into control and treatment groups in plastic containers with airtight lids. The containers used were narrow-opening gallon-sized containers with a rubber seal added to each cap to make it airtight and exclude oxygen during fermentation. For each pathogen, a 5-strain mix was inoculated into the treatment group containers at 1ml/L of cabbage juice and distilled water was added into the control group containers. Both control and treatment group containers were incubated at 25 °C for 15 days.

Microbial Analysis

Control and treatment groups were first enumerated approximately 30 min after inoculation. After that, enumeration was performed each day for the first 7 days and at 2-day intervals between 7 and 14 days. Samples from the control and treatment groups were enumerated on PCA (Plate Count Agar) to determine total viable count (TVC), and on MRS (de Man, Rogosa, and Sharpe) broth in an anaerobic environment for enumerating LAB. For treatments along with TVC and LAB count, we used PALCAM agar to enumerate *L. monocytogenes* and MacConkey Sorbitol agar to enumerate *E. coli* O157.

pH Measurements

Cabbage juice samples (10 ml) were removed from control and treatment groups to determine the pH using a pH meter (Oakton pH testr 30, calibrated with Oakton pH 4.01 buffer) each day for the first 7 days and at 2-day intervals between 7 and 14 days.

Data Analysis

The bacterial population was interpreted as the log CFU value per ml of the juice. Data points are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was analyzed using R; Duncan's new multiple range test was used to compare the significance of the differences in mean values at $\alpha = .05$. To compare mean values between the two pathogens studies, we used a Welch two sample *t*-test where $\alpha = .05$.

Results

pH

The pH for the control and treatment groups of sauerkraut inoculated with *L. monocytogenes* gradually decreased throughout the study period (Figure 1). The control and treatment groups both had a steady drop in pH for the first 7 days and had a relatively stable pH for the rest of the period—except at Day 15 for the control, which showed a higher drop in pH.

For the sauerkraut inoculated with *E. coli* O157, a similar trend of pH decline was observed (Figure 1). Similar to sauerkraut inoculated with *L. monocytogenes*, the pH stayed relatively stable after the 7-day period. Also, it was observed that in both treatment groups, the pH stayed slightly higher than the control groups. This finding might be attributed to some competitive growth of pathogens in the sauerkraut.

Total Viable Count and Lactic Acid Bacteria Count

Figure 2 shows the log growth of uninoculated controls for both total viable bacterial counts enumerated aerobically on PCA and LAB enumerated on MRS agar. TVC bacteria and LAB grew rapidly between Time 0 and Day 2. LAB for both control group fermentations were between 2 and 3 logs at Time 0 and peaked between 8 and 11 logs within 2–5 days. After that time, LAB counts dropped to approximately 4–5 logs at Day 15.

FIGURE 1

pH of Cabbage Inoculated With *Listeria monocytogenes* and *E. coli* O157 Fermented at 25 °C

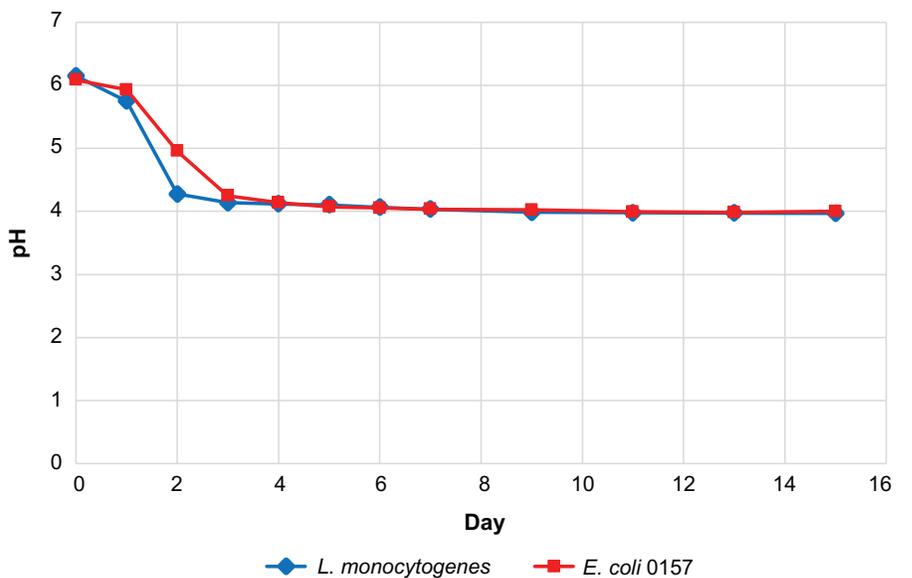
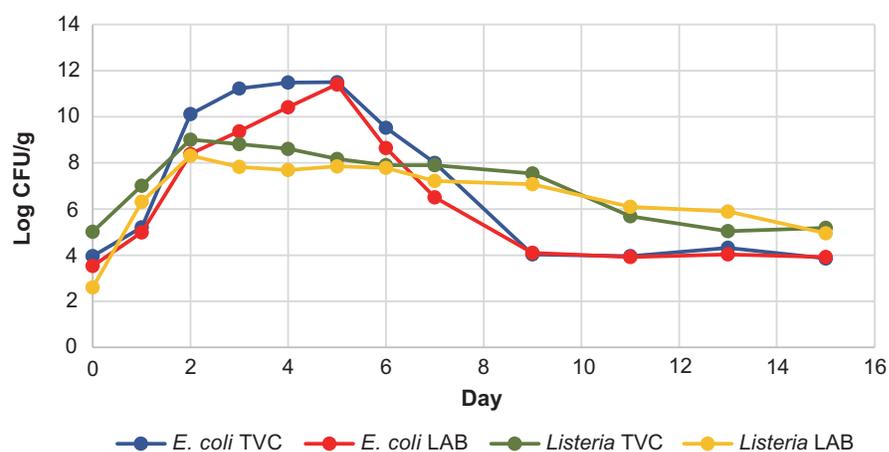


FIGURE 2

Total Viable Count (TVC) and Lactic Acid Bacteria (LAB) Count in Cabbage Fermented at 25 °C



Note. Data are presented as the mean value of three replications representing natural biota growth without the addition of pathogens.

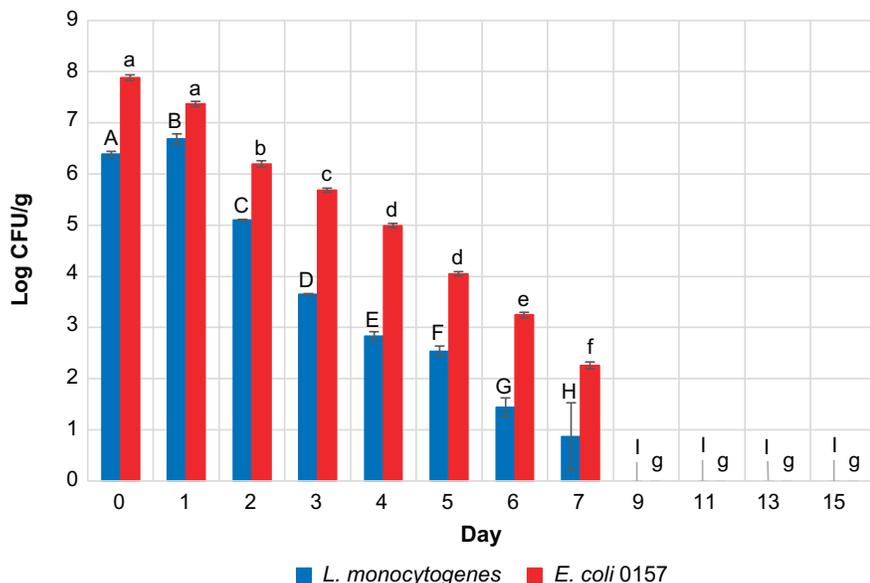
Lethality of *L. monocytogenes* and *E. coli* O157

The inoculum level of *L. monocytogenes* for the treatment group was 6.39 log CFU/g of

sauerkraut (Figure 3). The *L. monocytogenes* count slightly increased on Day 1, following a significant decrease in the number of pathogens from Day 2 until Day 7 ($p < .05$).

FIGURE 3

Lethality (Log CFU/g) of *Listeria monocytogenes* and *E. coli* O157 in Sauerkraut Fermented at 25 °C



Note. Data are presented as the mean value of three replications ± standard deviation.

^{a-g} Data points for *E. coli* O157 followed by the same lowercase letter for each day are not significantly different ($p \geq .05$).

^{A-H} Data points for *L. monocytogenes* followed by the same uppercase letter for each day are not significantly different ($p \geq .05$).

At 7 days, a 5-log reduction was observed in the treatment group and no pathogens were detected after Day 9.

In the sauerkraut inoculated with *E. coli* O157, the inoculum level was 7.88 log CFU/g of sauerkraut (Figure 3). There was a significant decrease in the *E. coli* O157 count from Day 1 until Day 7 ($p < .05$) and a 5-log reduction was observed at Day 7, with no detectable CFU/g after Day 9.

Discussion

The decreasing numbers of *L. monocytogenes* seen in the study are consistent with the study conducted by Niksic et al. (2005) that showed a gradual decrease in *L. monocytogenes* in sauerkraut fermented at 22 °C. Additionally, for the treatment group inoculated with *E. coli* O157, the rate of decrease in the number of pathogens over time was similar to studies by Arias et al. (2001) and Niksic et al. where a sig-

nificant decrease in the count of *E. coli* O157 was observed in sauerkraut fermented at 22 °C.

The inoculum level for both the pathogens was significantly different ($p < .05$) from the start of the studies (Arias et al., 2001; Niksic et al., 2005) and the number of pathogens in the following days followed the same pattern until Day 6. On Day 7, the number of pathogens was not significantly different and no pathogens were detected from Day 9, which suggests that despite the number of pathogens inoculated, there is a lethal effect when the pH drops to a certain level. In both studies, the decrease in pH coincided with the decrease in pathogen count, indicating the natural fermentation process in the sauerkraut that increased the acidity of the product had a negative effect on the survival of the pathogens.

Conclusion

Our study suggests that naturally fermented sauerkraut does not permit growth or survival of *L. monocytogenes* and *E. coli* O157. Naturally present LAB in the sauerkraut create a competitive environment for the pathogens, and the acidity produced by those LAB produces a lethal effect on these pathogens. Moreover, our study supports the safety of sauerkraut during the natural fermentation process at ambient temperature. These data can be used to support a HACCP approach applied to the process by retail and food service operators. 🐷

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References

- Arias, M.L., Monge-Rojas, R., Chaves, C., & Antillón, F. (2001). Effect of storage temperatures on growth and survival of *Escherichia coli* O157:H7 inoculated in foods from a neotropical environment. *Revista de Biología Tropical*, 49(2), 517–524. https://tropicalstudies.org/rbt/attachments/volumes/vol49-2/10-Arias_Effect.pdf
- Beganović, J., Kos, B., Leboš Pavunc, A., Uroić, K., Jokić, M., & Šušković, J. (2014). Traditionally produced sauerkraut as source of autochthonous functional starter cultures. *Microbiological Research*, 169(7–8), 623–632. <https://doi.org/10.1016/j.micres.2013.09.015>
- Breidt, F., Jr. (2005). Safety of minimally processed, acidified, and fermented vegetable products. In G.M. Sapers, J.R. Gorny, & A.E. Yousef (Eds.), *Microbiology of fruits and vegetables* (1st ed., pp. 313–335). CRC Press. <https://doi.org/10.1201/9781420038934>
- Breidt, F., & Fleming, H.P. (1998). Modeling of the competitive growth of *Listeria monocytogenes* and *Lactococcus lactis* in veg-

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- etable broth. *Applied and Environmental Microbiology*, 64(9), 3159–3165. <https://journals.asm.org/doi/pdf/10.1128/AEM.64.9.3159-3165.1998>
- Cotter, P.D., & Hill, C. (2003). Surviving the acid test: Responses of Gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews*, 67(3), 429–453. <https://doi.org/10.1128/MMBR.67.3.429-453.2003>
- Gandhi, M., & Chikindas, M.L. (2007). *Listeria*: A foodborne pathogen that knows how to survive. *International Journal of Food Microbiology*, 113(1), 1–15. <https://doi.org/10.1016/j.ijfoodmicro.2006.07.008>
- Hsin-Yi, C., & Chou, C.-C. (2001). Acid adaptation and temperature effect on the survival of *E. coli* O157:H7 in acidic fruit juice and lactic fermented milk product. *International Journal of Food Microbiology*, 70(1–2), 189–195. [https://doi.org/10.1016/S0168-1605\(01\)00538-4](https://doi.org/10.1016/S0168-1605(01)00538-4)
- Kim, J.K., D'Sa, E.M., Harrison, M.A., Harrison, J.A., & Andress, E.L. (2005). *Listeria monocytogenes* survival in refrigerator dill pickles. *Journal of Food Protection*, 68(11), 2356–2361. <https://doi.org/10.4315/0362-028x-68.11.2356>
- McDonald, L.C., Fleming, H.P., & Hassan, H.M. (1990). Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, 56(7), 2120–2124. <https://doi.org/10.1128/aem.56.7.2120-2124.1990>
- Niksic, M., Niebuhr, S.E., Dickson, J.S., Mendonca, A.F., Kozickowski, J.J., & Ellingson, J.L.E. (2005). Survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 during sauerkraut fermentation. *Journal of Food Protection*, 68(7), 1367–1374. <https://doi.org/10.4315/0362-028x-68.7.1367>
- Park, T.-K. (2017). Essential ingredients—Kimchi's beginnings: Bae-chu, the Chinese cabbage. *Koreana*. <https://www.koreana.or.kr/koreana/an/arn/selectArNttInfo.do?bbsId=1109&nnttSn=50947>
- Pérez-Díaz, I.M., Breidt, F., Buescher, R.W., Arroyo-López, F.N., Jiménez-Díaz, R., Garrido-Fernández, A., Bautista-Gallego, J., Yoon, S.-S., & Johanningsmeier, S.D. (2013). Chapter 51: Fermented and acidified vegetables. In Y. Salfinger & M.L. Tortorello (Eds.), *Compendium of methods for the microbiological examination of foods*. APHA Press. <https://doi.org/10.2105/MBEF0222.056>
- Plengvidhya, V., Breidt, F., Jr., Lu, Z., & Fleming, H.P. (2007). DNA fingerprinting of lactic acid bacteria in sauerkraut fermentations. *Applied and Environmental Microbiology*, 73(23), 7697–7702. <https://doi.org/10.1128/AEM.01342-07>
- Shin, J., Yoon, K.-B., Jeon, D.-Y., Oh, S.-S., Oh, K.-H., Chung, G.T., Kim, S.W., & Cho, S.-H. (2016). Consecutive outbreaks of enterotoxigenic *Escherichia coli* O6 in schools in South Korea caused by contamination of fermented vegetable kimchi. *Foodborne Pathogens and Disease*, 13(10), 535–543. <https://doi.org/10.1089/fpd.2016.2147>
- Shrestha, S., Grieder, J.A., McMahon, D.J., & Nummer, B.A. (2011a). Survival of *Listeria monocytogenes* introduced as a post-aging contaminant during storage of low-salt cheddar cheese at 4, 10, and 21 °C. *Journal of Dairy Science*, 94(9), 4329–4335. <https://doi.org/10.3168/jds.2011-4219>
- Shrestha, S., Grieder, J.A., McMahon, D.J., & Nummer, B.A. (2011b). Survival of *Salmonella* serovars introduced as a post-aging contaminant during storage of low-salt cheddar cheese at 4, 10, and 21 °C. *Journal of Food Science*, 76(9), M616–M621. <https://doi.org/10.1111/j.1750-3841.2011.02430.x>
- Steinkraus, K.H. (2002). Fermentations in world food processing. *Comprehensive Reviews in Food Science and Food Safety*, 1(1), 23–32. <https://doi.org/10.1111/j.1541-4337.2002.tb00004.x>
- Taormina, P.J. (2010). Implications of salt and sodium reduction on microbial food safety. *Critical Reviews in Food Science and Nutrition*, 50(3), 209–227. <https://doi.org/10.1080/10408391003626207>
- U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration. (2017). *Food code: 2017 recommendations of the United States Public Health Service, Food and Drug Administration*. <https://www.fda.gov/media/110822/download>

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