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Effect of stabilizers and surface materials on anti-listeria efficiency of hydrogen peroxide

Wirunwith, B., Racha, T. and Peamsuk, S.*

Food science and Technology Department, Thammasat University 99 Paholyothin Rd., Klong luang, Pathumthani, 12120, Thailand.

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Abstract The results demonstrated that the combination of H_2O_2 and stabilizers led to higher residual H_2O_2 levels compared to the control after 48 hours. Regarding the anti-Listeria activity, H_2O_2 in the presence of ethylene glycol showed better efficacy against *L. monocytogenes* than those of H_2O_2 in the presence of sodium citrate and H_2O_2 alone. Furthermore, the antimicrobial efficacy against *Listeria* spp. was markedly augmented when UV-C irradiation and hydrogen peroxide (H2O2) were employed concurrently, demonstrating the promise of this approach for controlling *Listeria* spp. on food equipment surfaces such as PTFE and stainless steel.

Keywords: *Listeria monocytogenes*, Stainless steel, PTFE, UV-C

Introduction

Listeria monocytogenes, a pathogenic bacterium, is of significant concern in the food industry due to its role in causing the infection known as listeriosis. Listeriosis can lead to severe illnesses, including sepsis, meningitis, or encephalitis, with potential lifelong harm and even fatal outcomes. Those most susceptible to severe illness are the elderly, fetuses, newborns, and individuals with compromised immune systems. In pregnant women, listeriosis can result in stillbirth, spontaneous abortion, and commonly, preterm birth. Moreover, listeriosis can manifest as mild gastroenteritis and fever in anyone (Bahrami *et al.*, 2020; Lecuit, 2020; Halbedel *et al.,* 2020). Within the food industry, certain strains of *L. monocytogenes* exhibit the ability to adapt and persist in various environmental conditions. They can survive under varying conditions such as temperature and relative air humidity. *L. monocytogenes* can persist on various surfaces, including those in direct contact with food and those not in contact with food, such as pipes, soil, and processing equipment, including meat slicers. This persistence is a significant concern, as *L. monocytogenes* on any surface can lead to contamination at various points in the food processing cycle, either

[*]**Corresponding Author:** Peamsuk, S.; **Email:** speamsuk@tu.ac.th

through direct contact or by cross-contamination of raw materials in processing plants (Zoz *et al*., 2022). Some bacteria possess the capability to adapt to and endure stressful conditions. To inhibit this persistence, the food industry employs a range of sanitizing methods, including the use of chemical agents (e.g., acidic, alkaline, enzymatic agents, or hydrogen peroxide), electromagnetic radiation (e.g., UV-C), and thermal processing. Typically, sanitizers such as hydrogen peroxide (H_2O_2) are used for inhibiting of *L. monocytogenes* in food industry (Yun *et al.,* 2012; Murray *et al.,* 2015). Some bacteria possess the capability to adapt to and endure stressful conditions. To inhibit this persistence, the food industry employs a range of sanitizing methods, including the use of chemical agents (e.g., acidic, alkaline, enzymatic agents, or hydrogen peroxide), electromagnetic radiation e.g., UV-C, and thermal processing. Typically, sanitizers such as H_2O_2 are used to inhibit *L. monocytogenes* growth (Yun *et al.,* 2012; Murray *et al*., 2015).

Hydrogen peroxide (H_2O_2) is a commonly sanitizer used in the food industry, particularly for cleaning and sterilization, owing to its broad-spectrum antimicrobial properties. It damages bacterial cells, proteins, and cell membranes effectively (Chihara *et al*., 2018). However, hydrogen peroxide is an unstable substance that can decompose via oxidation reactions into water, hydrogen, and free radicals, thereby reducing its antimicrobial efficiency. This necessitates the preparation of fresh solutions, which can be inconvenient and lead to increased chemical waste when surplus solution remains. To maintain the antimicrobial efficacy of hydrogen peroxide, stabilizing methods are required. Reported substances that can maintain H_2O_2 stability include sodium citrate and ethylene glycol, among others.

Additionally, using of electromagnetic radiation, especially UV-C at 254 nm, is employed to control *L. monocytogenes* and other strains. UV-C can penetrate bacterial cells, and nucleic acids (DNA and RNA) within these cells absorb UV-C at 254 nm (λmax). This absorption leads to the formation of pyrimidine dimers, causing DNA damage that hinders DNA replication. In this study, we investigated the stability of H_2O_2 and its anti-listerial activity on PTFE tubes and stainless-steel surfaces. Furthermore, the stabilization of H_2O_2 using ethylene glycol and sodium citrate was explored. Additionally, the ability of UV-C to reduce *L. monocytogenes* contamination on PTFE tubes and stainless-steel surfaces was assessed.

Materials and methods

Solutions preparation

A 1.0% (w/v) hydrogen peroxide solution was prepared from a 35.0% (w/v) hydrogen peroxide stock solution, which was diluted with sterile distilled water in sterile glass test tubes. The test tubes were wrapped in aluminum foil to protect from light and sealed with a biocap. Ten milliliters of the 1.0% (w/v) hydrogen peroxide solutions were then stored in an acrylic chamber measuring 30 x 30 x 50 cm and lined with black velvet (Figure 1). Each treatment was exposed to UV-C radiation at 253 nm.

Determination of hydrogen peroxide

The remaining hydrogen peroxide concentration was determined by redox titration with potassium permanganate ($KMNO_4$). The endpoint of the titration was used to calculate the concentration of hydrogen peroxide at 6, 12, 24, and 48 hours after stabilization with sodium citrate and ethylene glycol.

Surface material and equipment

PTFE tubes with outer and inner diameters of 5 and 3 mm, respectively, and a length of 2.0 cm. as well as 304 stainless steel sheet size of 2.5 x 2.5 cm were employed.

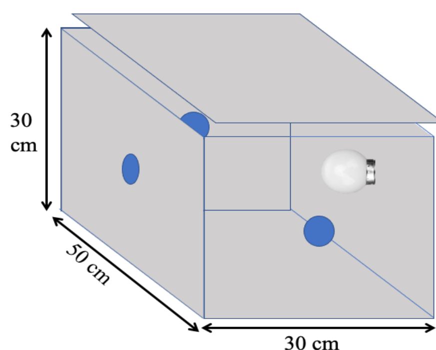

Figure 1. Acrylic chamber diagram

Stabilization of hydrogen peroxide

A 1% (w/v) hydrogen peroxide solution was stabilized with 2,600 ppm of sodium citrate (Watts *et al.*, 2007) and 2,500 ppm of ethylene glycol + 5,000 ppm

of $CuSO_4.5H_2O$ (Yazici, 2017) as a stabilizer, a 1% (w/v) hydrogen peroxide solution was used as a control. Those solution were stored in the dark at ambient temperature were taken at 6, 12, 24 and 48 hours for use in subsequent experiments.

Preparation of L. monocytogenes culture

L. monocytogenes strain DMST 17303 was obtained from Department of Medical Sciences, Ministry of Public Health (Nonthaburi, Thailand). Stock culture was maintained in tryptic soy broth supplemented with 0.6% (w/v) yeast extract (TSB-YE) (BD Difco, Sparks, MD, USA) containing 25% (w/v) glycerol in cryotube stored at -80 °C. A single colony of *L. monocytogenes* on trypticase soy agar (BD Difco, Sparks, MD, USA) supplemented with 0.6% (w/v) yeast extract (TSA-YE) prepared with trypticase soy agar (BD Difco, Sparks, MD, USA) supplemented with 0.6% (w/v) yeast extract (BD Difco, Sparks, MD, USA) was transferred to Erlenmeyer flask containing 50 mL of TSB-YE and incubated at 37°C, 18 hr. The culture was centrifuged at 4000 rpm for 15 min at 4°C, then washed with phosphate buffer solution twice, and resuspended in 10 mL phosphate buffer solution. The final concentration of *L. monocytogenes* solution was approximately 9.0 Log_{10}CFU/mL. H_2O_2 was added directly to test tubes containing of *L. monocytogenes* with cells population 6.0 Log_{10}CFU/mL after centrifuge under previously mentioned conditions.

Study of anti-listeria activity

L. monocytogenes culture was used for study anti-listeria activity by replaced of phosphate buffer solution with hydrogen peroxide stabilized with sodium citrate and ethylene glycol, followed by exposed of culture cells with H_2O_2 solutions each treatment for 10 min following the method outlined by Yun *et al*., 2012. The survival cell of *L. monocytogenes* after treatment with each solution was determined by transferring of 1 mL of cell suspension into phosphate buffer (pH 7.0) + 5 g/L sodium thiosulfate for neutralize of H_2O_2. Serially ten-fold dilutions were performed, and colony counting was conducted using the spread plate technique on TSA-YE after and incubated at 37ºC for 24 hours.

For simulation of contamination of *L. monocytogenes* on strainless steel, a cell suspension with log 6 CFU/ml was drop on strainless steel coupon and place until completely dry (5 minutes). Subsquenly H_2O_2 solutin were applied to the contamination area for treatment. For UV treatment, stainless steel coupon contaminated with *L. monocytogenes* were place in patri dish and expose to UV-

C light for 1 minute in the chamber (figure 1), followed by counting of survival cells.

Statistical analysis

The experiments were conducted with triplicates. Data were analyzed by SPSS statistics 20 software to perform the variance analysis (ANOVA) at a significant value of 0.05.

Results

Result showed the effect of stabilizer on the decomposition of 1.0% (w/v) H_2O_2 (Figure 2). The control solution, without the use of stabilizer, exhibited a downtrend of H_2O_2 remaining, which decreased to levels lower than 0.5% (w/v) after storage at room temperature for 6 hours. The rate of decrease was particularly high in the first 6 hours, followed by a slower decrease. The H_2O_2 values after storage for 12 hours were 0.4%, and for 24 and 48 hours, they were 0.3% (w/v), respectively. In terms of half-life, for H_2O_2 stored at ambient temperature, as shown in Figure 1, the hydrogen peroxide half-life was approximately 7 hours. Next, we explored the use of sodium citrate and ethylene glycol as H_2O_2 stabilizers. It demonstrated that the values of H2O2 remaining were higher than those in the control. In H2O2 solutions stabilized with sodium citrate, the H_2O_2 values were 0.6%, 0.5%, 0.5%, and 0.5% (w/v) at 6, 12, 24, and 48 hours, respectively (Figure 1). Additionally, in H_2O_2 solutions stabilized with ethylene glycol, the H_2O_2 values were 0.7%, 0.6%, 0.5%, and 0.5% (w/v) at 6, 12, 24, and 48 hours, respectively. The addition of sodium citrate to H_2O_2 increased the half-life to 12 hours. Moreover, ethylene glycol extended the H_2O_2 half-life up to 48 hours.

The anti-listeria activity of 1.0% (w/v) H_2O_2 solution stored at room temperature 6-48 hour at 10 mins exposure times is shown in Figure 3. In this study *L. monocytogenes* DMST17303 was completely destroyed when exposed to H_2O_2 concentration higher than 1.0% as a result show only 1.0% (w/v) of H_2O_2 solution. After *L. monocytogenes* with 6.0 Log_{10}CFU/mL initial population expose with control solution storage at 6 hour the solution cloud be destroyed of *L. mono-cytogenes* 4 log CFU/ml, and no survival cell were found in the H_2O_2 solution stabilize with sodium citrate and ethylene glycol. For 12 hours found a survival cell $5x10^2$ CFU/ml and $1x10^1$ CFU/ml for control and H_2O_2 stabilize with sodium citrate.

It showed that the anti-listeria of of 1% (w/v) H_2O_2, ethylene glycol, sodium critate, H_2O_2 stabilize with ethylene glycol, H_2O_2 stabilize with sodium

critate, UV-C and H_2O_2 combined with UV *L. monocytogenes* on stainless steel (Figure 4). *L. monocytogenes* population was reduced by 3 log/CFU withan initial load 6 log/CFU after treatment with 1% (w/v) H_2O_2. The stabilizers (EG and SC) also exhibited anti-listeria activity, reducing the cell population 1-2 log/CFU. When H_2O_2 was used in combination with stabilizer (H2O2+EG and H2O2+SC), a greater anti-listeria activity than with H_2O_2 alone was observed. UV treatment reduced of *L. monocytogenes* by 5 log/CFU and complete destruction occurred when exposed to H_2O_2 combined with UV.

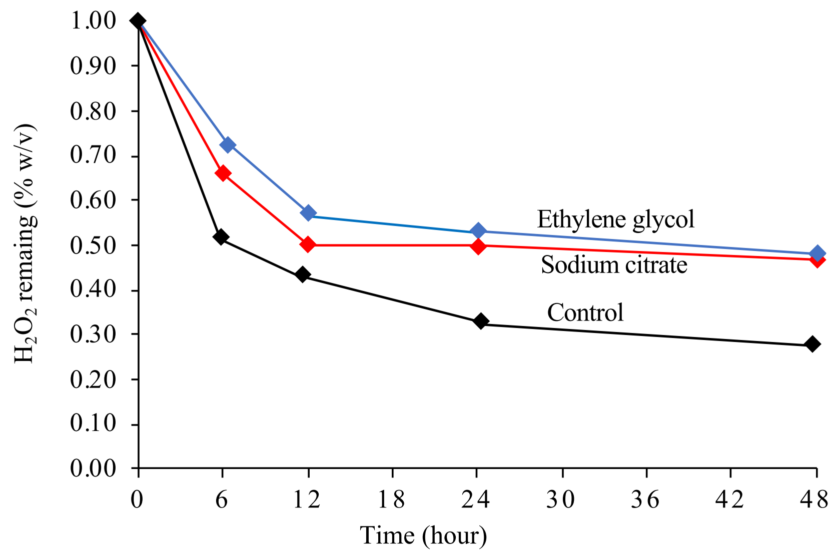

Figure 2. H_2O_2 remaining after storage at room temperature

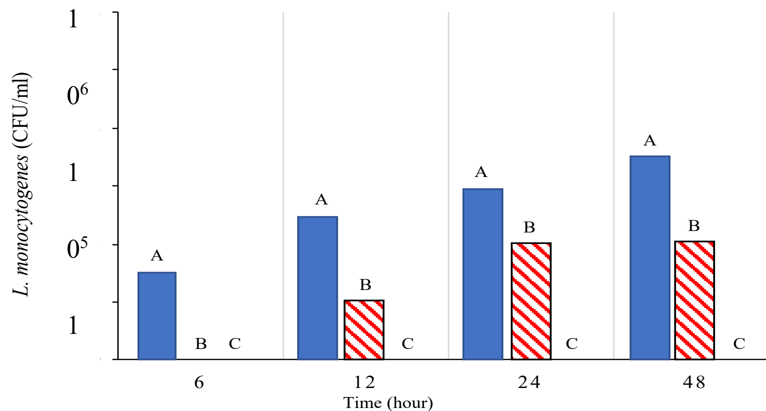

Figure 3. Anti-listeria activity of hydrogen peroxide, A; H_2O_2 solution (control), B; H_2O_2 solution stabilize with sodium citrate, C; H_2O_2 solution stabilize with ethylene glycol

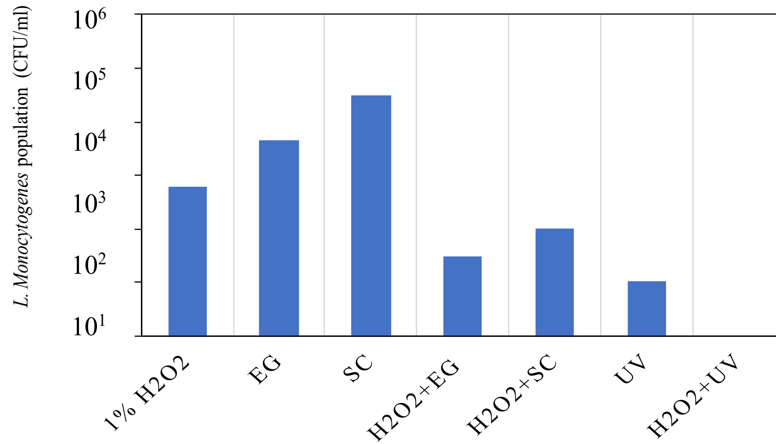

Figure 4. Anti-listeria activity of 1% (w/v) H_2O_2, ethylene glycol (EG), sodium critate (SC), H_2O_2 + ethylene glycol (H2O2+EG), H_2O_2 + sodium critate (H2O2+SC), UV-C (UV) and H_2O_2 +UV *L. monocytogenes* on stainless steel

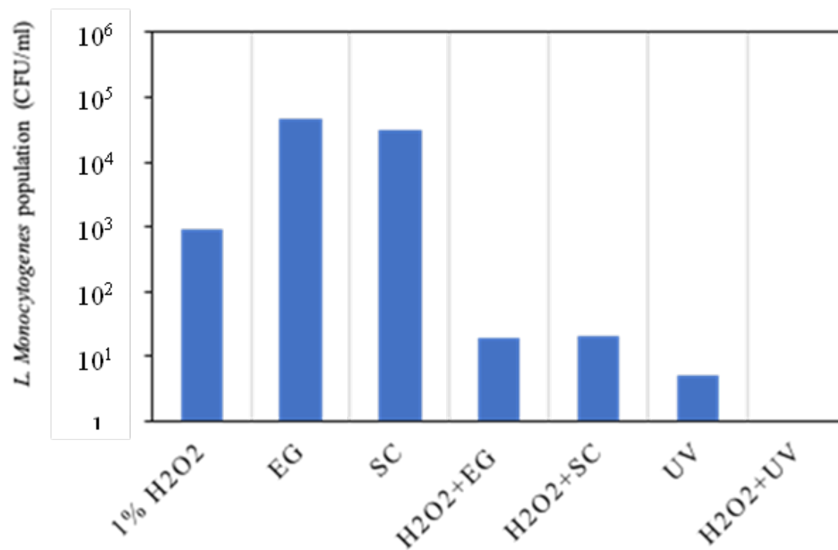

Figure 5. Anti-listeria activity of 1% (w/v) H_2O_2, ethylene glycol (EG), sodium critate (SC), H_2O_2 + ethylene glycol (H2O2+EG), H_2O_2 + sodium critate (H2O2+SC), UV-C (UV) and H_2O_2 +UV *L. monocytogenes* on PTFE tube

It showed that the anti-listeria activity of of 1% (w/v) H_2O_2, ethylene glycol, sodium critate, H_2O_2 stabilize with ethylene glycol, H_2O_2 stabilize with sodium critate, UV-C and H_2O_2 combined with UV *L. monocytogenes* on PTFE

tube (Figure 5). The result was similar to those observed on starinless steel, with a reduction of 3 log/CFU from initial load of 6 log/CFU after treatment with 1% (w/v) H_2O_2 and stabilizer (EG and SC). However, sodium citrate is shown similar of level of anti-listerial activity to ethylene glycol, reducing the population by 1 log/CFU. When H_2O_2 was used in combination with stabilizer (H2O2+EG and H2O2+SC), a greater anti-listeria activity than H_2O_2 without stabilizer was observed. Finally, UV treatment can be reduced of *L. monocytogenes* 5 log/CFU and complete destruction occurred when exposed to H_2O_2 combined with UV.

Discussion

Result indicated that H_2O_2 undergose self-decomposition (Daneshvar *et al*., 2008: Pędziwiatr, 2018), the remaining H_2O_2 concentration decreased after storage at room temperature, reducing more than 50% of its initial concentration due to oxidizing activity of hydrogen peroxide. Additionally, H_2O_2 can be regenerated though the reaction of •OOH two molecules in termination processes of oxidation reaction, thereby fully compensating for H_2O_2 in system (Cataldo, 2014: Chuand and Anastasio, 2008). Futhermore, the use of sodium citrate and ethylene glycol increases the stability of H_2O_2 by anti-oxidation properties. This result decreased rate of H_2O_2 decompositon with oxidation reaction, wherein oxygen-oxygen bond breaks, leading to the decomposes of H_2O_2 into water and oxygen.

The anti-listeria activity of H_2O_2 solution was evaluated. The strain used in this experiment displayed greater sensitivity compared to other strains used in the research by Yun *et al*., 2012. They also observed the survival of *L. monocytogenes* cells when exposed to 1% (w/v) H_2O_2 20 mins. H_2O_2 stabilize with sodium citrate exhibited grater anti-listeria activity than the control (1% H_2O_2 without stabilizer). This enchanment can be attributed to sodium citrate ability to maintain H_2O_2 concentration and its antimicrobial properties, and acidity. Additionally, H_2O_2 with ethylene glycol displayed supeerior anti-listeria activity compared to both control and sodium citrate as a stabilizer across all storage times**.** This is because ethylene glycol is a toxic alcohol that exhibits toxicity to bacteria cell (Iqbal *et al*., 2022).

Moreover, bacteria can neutralize low concentration of H_2O_2 through the enzyme catalase. However, *L. monocytogenes* effectively inhibited by high concentration of H_2O_2, especially when sodium citrate and ethylene glycol were used as stabilizers. In addition, UV-C is a electromagnetic radiation that can be penetrated bacteria cell DNA, which absorbed by UV light at 254 nm. UV leads to destruct DNA structure, inhibit of DNA replication, and untimately results in bacteria death. To summarize, it is indicated that sodium citrate and ethylene

glycol can effectively maintain of H_2O_2 stability and enhance their anti-listeria activity. The finding demonstated that H_2O_2 and UV-C can reduce *L. monocytogenes* contaminated on PTFE tube, with the degree of reduction dependent on the material type. Combining H_2O_2 and UV-C can enhance anti-listerial activity and effectively control bacterial contamination on surfaces, thereby improving food safety.

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