
Predictive Modelling for Inactivation of *Escherichia coli* Biofilm with Combined Treatment of Thermosonication and Organic Acid on Polystyrene Surface

[Emel Unal Turhan](#)^{*} and Esabil Emrah Koca

Posted Date: 8 November 2024

doi: 10.20944/preprints202411.0610.v1

Keywords: biofilm; *Escherichia coli*; thermosonication; organic acid; multiple linear regression



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Article

Predictive Modelling for Inactivation of *Escherichia coli* Biofilm with Combined Treatment of Thermosonication and Organic Acid on Polystyrene Surface

Emel Unal Turhan * and Esabil Emrah Koca

Department of Food Technology, Faculty of Kadirli Applied Sciences, Osmaniye Korkut Ata University, 80760, Kadirli, Osmaniye, Turkey

* Correspondence: emelunalturhan@gmail.com

Abstract: The purpose of this study was to determine antibiofilm effect of combined sonication treatment with organic acid on polystyrene surface and to develop a predictive model for *Escherichia coli* biofilm inactivation. Polystyrene plates containing *E. coli* biofilm were exposed to sonication with different inactivation solutions (PBS, lactic acid and acetic acid) at different temperatures (20, 40 and 50°C) and times (2 and 5 minutes). The effects of temperature, time and inactivation solution on *E. coli* biofilm removal were found to be statistically significant ($p < 0.05$). With the use of organic acids, increasing treatment time and temperature, viable cell counts and optical density of *E. coli* biofilms significantly decreased by 0.43-6.21 log CFU/mL and by 0.13-0.72 (OD600), respectively ($p < 0.05$). The highest *E. coli* biofilm inactivation was achieved by the combination treatment of organic acid and thermosonication at 50°C for 5 minutes. A significantly positive correlation was found between test methods based on viable cell count and optical density measurement. According to multiple linear regression analysis, R^2 values of prediction models for biofilm inactivation in terms of viable cell count and OD were 0.84 and 0.80, respectively. Predictive model developed by using viable cell count data were suggested for food industry and processors due to more accuracy.

Keywords: biofilm; *Escherichia coli*; thermosonication; organic acid; multiple linear regression

1. Introduction

Biofilms are defined as aggregates of microbial cells embedded within called extracellular polymeric substances. Several foodborne pathogens can form biofilms on food contact surfaces depending on several factors, such as temperature, pH, nutrients content, salinity, contact surface properties, and microorganism strain [1]. Especially, *Escherichia coli* O157:H7 biofilm is a serious threat to food safety and public health since the infectious dose of *E. coli* O157:H7 is low (<100 cells). *E. coli* can colonize food processing surfaces including plastic, glass, granite, marble, stainless steel, wood and siltstones. Cross-contamination of foods by food-contact surfaces harbouring low numbers of the pathogen can potentially lead to outbreaks [2]. Foodborne pathogen biofilms are the reason of approximately 60% of the world's outbreaks, thus the presence of bacterial biofilms in food industry is a major concern and poses risks to food safety [3]. Foodborne pathogens that have formed biofilm on food-contact surfaces increase the potential for food cross-contamination. Cleaning and sanitising food contact surfaces are essential to prevent cross-contamination and the spread of foodborne illnesses. However, removing of biofilm is a very challenging because the biofilms of pathogens were more resistant to hurdles than their planktonic cell. EPS matrix of biofilms provides compositional support and protection of microbial communities against the harsh environments [4].

Current strategies for controlling pathogen biofilms are based on physical (heating, ultrasonication, UV-V, irradiation and cold oxygen plasma) and chemical (chlorine, hydrogen peroxide, ethanol and organic acid) inactivation methods [5]. In recent years, there has been a

growing trend towards environmentally friendly and safe treatments to inactivate biofilms [6]. As an emerging technology, ultrasound is believed to be a promising technology for effectively detaching the biofilms from contact surfaces and leading to a bactericidal effect simultaneously [7]. In particular, an effective biofilm reduction provided with combined treatment of ultrasound and organic acids. The most used organic acids are acetic acid, lactic acid, citric acid, malic acid and peracetic acid in food industry. Undissociated form of organic acids and their pH are responsible for antimicrobial activity [8]. When combined with organic acids to remove biofilms, ultrasound can expose inner cells to the disinfectants and promote the permeability of chemical disinfectants into the biofilms by mechanical oscillation, helping chemical sanitisers to achieve an enhanced bactericidal effect on biofilms [4]. All factors used in food preservation are defined as “barriers” and a wide variety of barriers are applied in food preservation. Potential barriers used in food preservation can be divided into physical and chemical barriers as mentioned before. In fact, for more effective microbial inactivation in food preservation, the use of more than one barrier, i.e. the combined use of barriers, has been resorted to. In biofilm inactivation, both physical and chemical barriers can be applied in combination and a stronger decontamination can be achieved [9]). When ultrasound is applied alone, its inactivation effect on various pathogens varies between 0.5-1.98 log CFU/g. However, when it is applied together with other disinfection methods (organic acids, alcohols, essential oils, bacteriocins, UV, etc.), this effectiveness increases [10].

Ultrasonic processes have been usually performed at low temperatures. However, in recent years, thermosonication has been applied with a greater effect on inactivation of microorganisms than heat alone [11]. It combines mild heat of 37 to 75 °C with low frequency ultrasound waves (20 kHz) treatment [12]. Thermosonication applications in food industry offer numerous advantages such as better product quality, shorter processing time, being eco-friendly and less hazards. Microbial inactivation mechanism of thermosonication is attributed to physical (cavitation and heat) and chemical (formation of free radicals) effects [11,13]. Single microbial control strategies are insufficient to completely remove pathogen bacteria with no damage to food contact surfaces or equipment surfaces. However combined treatment of thermosonication with chemical agents provides both more effective sanitation and prevents damage of food contact surface or equipment due to extremely intense treatment conditions (longer processing time, higher processing temperature and higher amount of antimicrobial agents). Food contact surfaces have been subjected to a shorter exposure time and lower amounts of disinfectants during thermosonication treatment [12]. Effects of treatment conditions on microbial inactivation are explained using predictive modelling. Various prediction models have been asserted for fitting the elimination data of foodborne pathogens, however they are case-dependent and affected by environmental factors, bacterial strain, and processing parameters [14]. Prediction of microbial inactivation with mathematical equations plays an important role in hazard analysis of critical control points and food safety. Only a few studies deal with predictive modelling of biofilm inactivation on sonication treatment. On the other hand, food industry requires these predictive models for effective sanitation procedure [15].

Thermosonication and its combined treatment have been employed to food products as a novel technology. However, data on inactivation applications using thermosonication are considered insufficient against foodborne pathogens [12]. To our knowledge, no prior study has reported biofilm inactivation with thermosonication on food contact surface. The aim of this study was to determine the effectiveness of combined treatments with thermosonication and organic acids on removal of *E. coli* biofilm on polystyrene surface.

2. Materials and Methods

2.1. Bacterial Strain, Culture Preparation and Organic Acids

Escherichia coli (NCTC 12241) was provided by the culture collection of Osmaniye Korkut Ata University (Osmaniye/Turkey) and stored in Tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) with 20% glycerol (Sigma-aldrich, Germany) at -20°C. Cells from stock were firstly streak plated on Tryptic Soy Agar (TSA; Difco) plate and grown for 24 hours at 37 °C. After that, a

single colony was grown overnight at 37°C in TSB. An overnight culture was used as a constant concentration of bacterial cells (approximately 8 log cfu/mL) for all experiments [16,17].

All inactivation treatment solutions were prepared immediately before each experiment and were used within 30 min. Treatment solutions including PBS (control), 2% lactic acid (v/v, Sigma-Aldrich, Austria) and 2% acetic acid (v/v, from glacial acetic acid-Merck-Germany) were prepared with demineralized water and kept at room temperature (approx. 20°C).

2.2. Biofilm Formation

Biofilm formation was performed according to microplate method. 3 mL of TSB with 1% overnight culture of *E. coli* strain were added to each well of a 12-well microplate and left incubation for 48 hours at 37°C. The medium containing planktonic cells was removed and each well was washed three times with 3.5 mL of phosphate buffered saline (PBS). The part remaining at the bottom of the wells after the washing procedures was evaluated as biofilm. Biofilms obtained in this section (2.2) were used in the following experiments (2.3, 2.4 and 2.5).

In order to be biofilm positive for a microorganism, the optical density result must be higher than the optical density results of the control samples (OD_{nk}: negative control optical density). In addition, *E. coli* is classified as strong ($4 \times \text{OD}_{\text{nk}} < \text{OD}$), medium ($2 \times \text{OD}_{\text{nk}} < \text{OD} \leq 4 \times \text{OD}_{\text{nk}}$), weak ($\text{OD}_{\text{nk}} < \text{OD} \leq 2 \times \text{OD}_{\text{nk}}$) and non-adherent ($\text{OD} \leq \text{OD}_{\text{nk}}$) according to their biofilm formation abilities [7].

2.3. Crystal Violet Assays of Biofilms and Optical Density

E. coli biofilms in well were stained with 3.5 mL %0.1 crystal violet for 30 min and washed thrice with 3.5 mL water for removal of unbound crystal violet. After drying, attached crystal violet was dissolved in 3.5 mL absolute ethanol and optical density was measured at 600 nm using the Elisa Microplate Reader (Rayto-RT ELISA Microplate Reader). On those cases where the absorbance (optical density) was higher than 1, the samples were diluted (1:10), values were then multiple by 10 to get the final result [18,19].

2.4. Count of Viable Cells in Biofilm of *E. Coli*

Biofilm cells were resuspended in 1 mL PBS by pipetting rigorously and serial diluted in PBS. Cells were plated on TSA (Difco). Plates were incubated at 37°C for 24-48 hours. After incubation period, viable cell counts of *E. coli* biofilms are reported as log CFU/MI [5,20].

2.5. Single and Combined Sonication Treatment against *E. coli* Biofilm

1% of overnight grown cultures at 30°C were inoculated in 12 well polystyrene microtiter plates containing 3 mL TSB at each well and then incubated at 37°C for 48 hours. Biofilms were washed once with PBS and expose to 3 mL treatment solution (PBS, 2% lactic acid or 2% acetic acid) in ultrasound bath (ISOLAB; Ultrasonic power: 360 W, frequency: 40 kHz; Heating power: 600 W) under different conditions (at 20, 40 and 50°C and for 2 and 5 min). After exposure to sonication and organic acids, biofilms of *E. coli* were washed with PBS. Biofilms remaining at the bottom of the wells after the washing procedures was evaluated with optical density measurement and with enumeration of viable cell counts. In the experiments, PBS was applied as a positive control and pathogen-free TSB was applied as a negative control [5,18,19].

2.6. Statistical Analysis and Predictive Modelling

All the experiments were run in triplicate. Data were analysed with SPSS version 15.0 (SPSS Inc., USA) statistical package. All the analysis results were given as mean \pm standard deviation (SD). Statistical differences ($p < 0.05$) were evaluated by Duncan's multiple comparison tests (variance analysis). The relationship between the results obtained in terms of optical density and viable cell count was explained with "Simple Linear Correlation". Additionally, mathematical multiple linear regression model was developed the prediction of biofilm inactivation [21].

In multiple linear regression model, the relationship between two or more independent variables and a dependent variable was explained by fitting a linear equation to observed data. The dependent variable (Y) is related to every independent variable (X). The population regression line for p independent variables $X_1, X_2, X_3, \dots, X_p$ is described to be $\mu Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \dots + \beta_p X_p$. It defines how the mean response μY varies with the independent variables. The observed values for y change about their means μY and are accepted to have the same standard deviation. The fitted values $b_0, b_1, b_2, \dots, b_p$, predict the parameters $\beta_0, \beta_1, \beta_2, \dots, \beta_p$ of the population regression line. Since the observed values for Y change about their means μY , the multiple regression model involves a term for this variation. In brief, the model is expressed as DATA = FIT + RESIDUAL, where the "FIT" term describes the expression $\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \dots + \beta_p X_p$. The "RESIDUAL" term describes the deviations of the observed values y from their means μy , which are normally distributed with mean 0 and variance. ϵ represents the notation for the model deviations [22]. Formally, the multiple linear regression model was given in Equation (1)

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \epsilon \quad (1)$$

In this study, the classical method was used for 3 independent variables (X_1 =temperature, X_2 =time and X_3 =solution) and 1 dependent variable (Y =decrease in cell number or optical density) and the above regression equation model was used (equation 1). This regression equation model made it possible to estimate the effect of sonication treatment at different conditions on *E. coli* biofilm inactivation. In general, the closer to 1 of the R^2 and adj. R^2 values, and the smaller RMSE (root mean square error) and SSE (The sum of squares error) values will imply a suitable fit for the data [23].

3. Results and Discussion

In this study, data related reduction in viable cell count, and optical density were used to determine the efficiency of single and combined sonication treatment on *E. coli* biofilm inactivation. This data explained correlation between OD analysis and viable cell analyses. Furthermore, a prediction model for *E. coli* biofilm inactivation was used according to data obtained after sonication treatments. Prediction equations estimate the probability of *E. coli* biofilm inactivation after sonication treatments under different time and temperature conditions.

3.1. *E. coli* Biofilm Formation on Microplates

Plastic and stainless steel are often used materials of food contact surfaces in food industry and households. Surface materials support the growth of biofilm in the following order: latex>polyethylene>PVC>polypropylene>stainless steel>glass. In the current study, biofilm detachment on polystyrene surface was examined while most of the data in the literature are about biofilm removing from stainless steel. Only a few studies focused on biofilm detachment from plastic, especially from polystyrene surface [24].

Table 1 shows data regarding *E. coli* biofilm formation on polystyrene microplates (Table 1). In our study, biofilms formed by *E. coli* were strong based on the optical density (OD600: 1.02) results. According to previous studies, the optical density of *E. coli* biofilms in microplates varied between approximately 0.50-2.00 [7,25–27]. Similar to the present data about *E. coli* biofilm formation, in a previous study, biofilm populations in various surfaces including stainless steel, glass, plastic (polyethylene) and wood were stated as 8.5, 8.8, 8.7 and 9.6 log CFU/coupon, respectively [2]. The ability of *E. coli* to form biofilms in microplates may vary depending on the live cell concentration, microbial growth phase, properties of microplates (number and size of wells, type of material, etc.), growth medium, incubation conditions, etc. [28]. For example, Nesse et al. [29] reported that *E. coli* can form biofilms on various food processing surfaces such as stainless steel, glass and polystyrene, but among these surfaces, stainless steel and polystyrene surfaces triggered higher amounts of biofilm formation compared to glass.

Table 1. *E. coli* biofilm formation on polystyrene microplates.

OD	Viable cell counts (log CFU/mL)	Biofilm formation
----	---------------------------------	-------------------

Biofilm	1.02 ±0.03	8.56 ±0.08	+
Negative control	0.14±0.00	0.00±0.00	-

3.2. Effect of Single Sonication and Combined Sonication Treatment against *E. coli* Biofilms

Simple decontamination procedure containing antimicrobial substances was not sufficient for the inactivation of high resistant biofilms. Therefore, some studies combined these compounds with sonication to enhance the bactericidal effectiveness. Food contact surfaces can be damaged mechanically after exposure to sonication or antimicrobial substances for long treatment time. Thermosonication, the combined treatment of heat and ultrasound, provides more effective sanitation in short time with minimal damage to food contact surface than power ultrasound [30–32]. Sonication treatment may effectively destruct the polysaccharide and protein from bacterial biofilm and change the protein composition of detached EPS. The polysaccharide in loosely bound EPS was usually the first barrier to protect microbial cells against the harmful impacts of chemical disinfectants. Without the protection of extracellular polysaccharide, microbial cells in biofilm were susceptible to disinfectants [33].

In this study, polystyrene plates containing *E. coli* biofilm were subjected to single sonication treatment with PBS and combined sonication treatment with lactic acid or acetic acid at 20, 40 and 50°C, for 2 and 5 min. After that, biofilm inactivation was evaluated according to viable cell counts and OD of *E. coli* biofilms remaining in plate. Results of viable cell count analysis was compatible with those of OD analysis and generally showed similar reduction trend with regard to *E. coli* biofilm inactivation. (Tables 2 and 3). Treatment temperature, treatment time and type of washing solutions (lactic acid, acetic acid and PBS) significantly influenced *E. coli* biofilm destruction ($p<0.05$). Stronger antibiofilm effect was observed with an increase in treatment time and treatment temperature. Additionally, combined treatment of sonication with organic acids caused higher biofilm reduction. The use of lactic acid or acetic acid contributed to an additional reduction between 1.5 and 3 log cfu/mL in *E. coli* biofilm on polystyrene surfaces as compared to the individual use of sonication. These findings were consistent with previous studies, which reported that microbial cells were more susceptible to combined sonication treatment than single sonication treatment [4,31,32,34,35]. It can be interpreted that a similar decontamination rate can be achieved by lowering the treatment time with combination treatment of thermosonication and organic acids.

Table 2. Reduction in viable cells of *E. coli* biofilm with combined and single ultrasonication treatment (log CFU/mL).

Sonication conditions	Control (PBS)	Lactic acid (2%)	Acetic acid (2%)
20°C – 2 min.	0.43±0.03 ^{eB}	2.18±0.20 ^{eA}	1.95±0.34 ^{dA}
40°C – 2 min.	1.12±0.13 ^{dB}	2.88±0.15 ^{dA}	3.01±0.08 ^{cA}
50°C – 2 min.	1.66±0.01 ^{cB}	4.06±0.14 ^{cA}	3.99±0.20 ^{bA}
20°C – 5 min.	1.05±0.26 ^{dB}	3.98±0.02 ^{cA}	3.94±0.09 ^{bA}
40°C – 5 min.	2.56±0.11 ^{bB}	5.74±0.04 ^{bA}	5.96±0.15 ^{aA}
50°C – 5 min.	3.37±0.19 ^{aB}	6.21±0.19 ^{aA}	6.14±0.26 ^{aA}

A-B: The difference between the values indicated with different letters in the same row is statistically significant ($p<0.05$). a-e: The difference between the values indicated with different letters in the same column is statistically significant ($p<0.05$).

Table 3. Reduction in optical density of *E. coli* biofilm with combined and single ultrasonication treatment (OD).

Sonication conditions	Control (PBS)	Lactic acid (2%)	Acetic acid (2%)
20°C – 2 min.	0.13±0.02 ^{eA}	0.15±0.03 ^{dA}	0.14±0.03 ^{eA}
40°C – 2 min.	0.23±0.00 ^{dA}	0.27±0.06 ^{cA}	0.29±0.01 ^{dA}
50°C – 2 min.	0.37±0.01 ^{cbC}	0.63±0.01 ^{bA}	0.54±0.01 ^{bB}
20°C – 5 min.	0.30±0.01 ^{cdC}	0.60±0.01 ^{bA}	0.45±0.05 ^{cB}

40°C – 5 min.	0.45±0.09 ^{bB}	0.66±0.02 ^{abA}	0.52±0.02 ^{bcAB}
50°C – 5 min.	0.70±0.03 ^{aA}	0.71±0.01 ^{aA}	0.72±0.04 ^{aA}

A-C: The difference between the values indicated with different letters in the same row is statistically significant ($p < 0.05$). a-e: The difference between the values indicated with different letters in the same column is statistically significant ($p < 0.05$).

Table 2 shows the reduction of *E. coli* biofilm in terms of viable cell counts after sonication treatment under different conditions. *E. coli* biofilm reduction varied from 0.43 to 6.21 log CFU/mL. The lowest and highest of *E. coli* biofilm reduction was obtained with single sonication at 20°C for 2 min and combined thermosonication treatment at 50°C for 5 min, respectively. Organic acid treatment was found to be statistically more effective than PBS treatment in terms of *E. coli* biofilm detachment ($p < 0.05$). However, type of organic acids (lactic acid or acetic acid) was not significantly effective on biofilm reduction ($p > 0.05$). This means that any of these organic acids can be used for removal of *E. coli* biofilm. Similarly, the impact of organic acid type on *E. coli* biofilm destruction was reported to be insignificant by Yuk et al. [36] and Stopforth et al. [37]. In this study, at all sonication conditions, highest antibiofilm effect was mostly exhibited with lactic acid despite of statistically insignificant. Similarly, Ji et al. [38] detected that lactic acid provided higher reduction in mature biofilm of *E. coli* than acetic acid.

Inactivation of bacteria with sonication is because of the damage of the cell wall, especially biofilm matrix and cytoplasmic membrane [23]. Sonication treatment partly influenced the outer layer of biofilm matrix, and the cells in the inner layer could be protected from the sanitizer, and so could survive [33]. The antibiofilm effect mechanism of organic acids was associated with their undissociated form and their pH. Undissociated organic acid molecules damages microbial cell membrane and thus lead to microbial inhibition. The dissociation of organic acids can change depending on time and temperature of ultrasonication. For example, dissociation may increase as the temperature rises and the sonication time is prolonged [39]. As a matter of fact, the present study conducted a maximum of ultrasonication at 50°C for 5 min since treatment with a higher temperature and time may trigger dissociation of organic acids.

The efficacy of decontamination technique depends on microbial load, sonication duration, temperature of treatment, intensity, frequency and so on [23]. For instance, Lee et al. [40] stated that a 5-log reduction of *E. coli* K12 was achieved with single thermosonication treatment at 61°C and for 4 min, whereas the same reduction of *E. coli* (5 log) was reached with combination treatment of thermosonication and pressure in less treatment time (0.075 min) combined with pressure treatment. In the study of Kwak et al. [30], *E. coli* O157:H7 was reduced 0.97 log CFU/g with thermoultrasound and calcium propionate (2%) treatment at 50°C for 10 min. Similarly, Turhan and Polat [41] reported that combined treatment of sonication and organic acids (lactic, acetic, malic and citric acid) created additional *E. coli* biofilm destruction with synergistic effect. In another study about combined sonication treatment, single ultrasound treatment (500kHz) and combined ultrasound treatment with nisin (500 kHz) caused approximately 1 and 2 log reduction of *E. coli* for 20 min, respectively [42]. Fan et al. [32] reported that thermosonication pretreatment (for 15 min at 55°C) enhanced the sporicidal activity of UV irradiation in suspension with an additional reduction between 2.74 and 3.78 log. Similar to all these previous studies, the present study confirmed that combined thermosonication treatment with other inactivation methods was more effective on microbial inactivation.

Table 3 gives the detachment of *E. coli* biofilm in terms of OD after sonication treatment under different conditions. With the use of organic acids, increasing treatment time and temperature, it was exhibited a greater detachment of *E. coli* biofilm on polystyrene plate ($p < 0.05$). Similar to results in terms of viable cell count, highest biofilm removal in terms of OD (decrease in optical density: 0.72 OD) was obtained with combination treatment of sonication and lactic acid for 5 minutes at 50°C. In the study of Park and Chen [25], *E. coli* biofilm on polystyrene plate was subjected to lactic acid and acetic acid for 20 min. In terms of OD, *E. coli* biofilm removal with lactic acid and acetic acid varied from 0.01 to 0.26 and from 0.03 to 0.21, respectively. In our study, the treatment of lactic acid and

acetic acid with sonication at 20°C for 5 min caused higher *E. coli* biofilm detachment (between 0.45 and 0.60 OD). In accordance with the results of viable cell counts analysis, these results based on OD measurement confirmed that the combination treatment of organic acid and sonication was more effective on biofilm detachment than single organic acid treatment. As mentioned above, the antibacterial mechanism of action of acetic acid and lactic acid is related to lowering the pH of the environment. The ability of these organic acids to lower the pH has also been associated with their dissociated and non-dissociated forms. Non-dissociated and uncharged organic acids are primarily responsible for the antibacterial effect. Another mechanism of antimicrobial action of organic acids has been stated as the “weak organic acid theory”. Particularly, lactic, acetic, malic, citric and propionic acids use this mechanism. For example, lactic acid disrupts membrane stability by reducing membrane-associated molecular interactions in gram-negative bacteria and promotes the formation of pores that cause rapid cell death. In addition, lactic and acetic acids have been reported to disrupt the transmembrane proton motive force, denature acid-sensitive proteins and DNA, and generally interfere with both metabolic and anabolic processes [43]. The lower pH environment and the presence of organic acid may be reduced resistance of *E. coli* to sonication [44]. Also, previous researchers confirmed that *E. coli* showed sensitivity to sonication with increased time and temperature of treatment [40,45–47].

3.3. Relationship between Biofilm Inactivation Tests

In previous studies, OD measurement was often applied for the detection of microbial inactivation efficiency [28,41]. OD analysis is faster than plate counting; however, it is based on turbidity it registers all bacteria (cell biomass), dead and alive [48,49]. Therefore, viable cell analyses based on only live cell are reported to give more accurate and safe results [50]. For instance, in a previous study, 68-86% *E. coli* biofilm inactivation in terms of viable cell counts and 52-60% *E. coli* biofilm removal in terms of OD was obtained after 2% organic acid treatment (malic acid, citric acid, gallic acid) for 5, 10 and 20 minutes. This confirmed that OD technique include all bacteria (dead and alive) [28]. Therefore, the present study evaluated biofilm inactivation in terms of viable cell count in addition to OD for more precise results about cell reduction. Furthermore, relationship between biofilm inactivation tests was explained with simple linear correlation analysis.

To detect the relationship between *E. coli* biofilm inactivation tests, a scatter plot was created using the observation values for x (OD) and y (viable cell count) variables (Figure 1). According to the results obtained from the scatter plot and simple linear correlation analysis data, a positive linear and significant relationship ($r:0.817$, $p<0.01$) was determined between variables of viable cell count and optical density. Plate counting and OD methods were compared to determine the microbial growth. The comparison demonstrated that OD may be used as an alternative technique to detect the viable cell count of microorganisms. Microbial growth rate can be deduced from the slope of the profiles obtained using OD. These results indicated that both of inactivation test methods based on viable cell count and OD were useful for comparison of sonication treatments. However, analysis methods based on viable cell counts were suggested since inactivation data obtained from viable cell counts achieved more accuracy. Similar to our results; Loske et al. [48] stated that a correlation between the data from the plate count method and turbidimetric analysis was obtained.

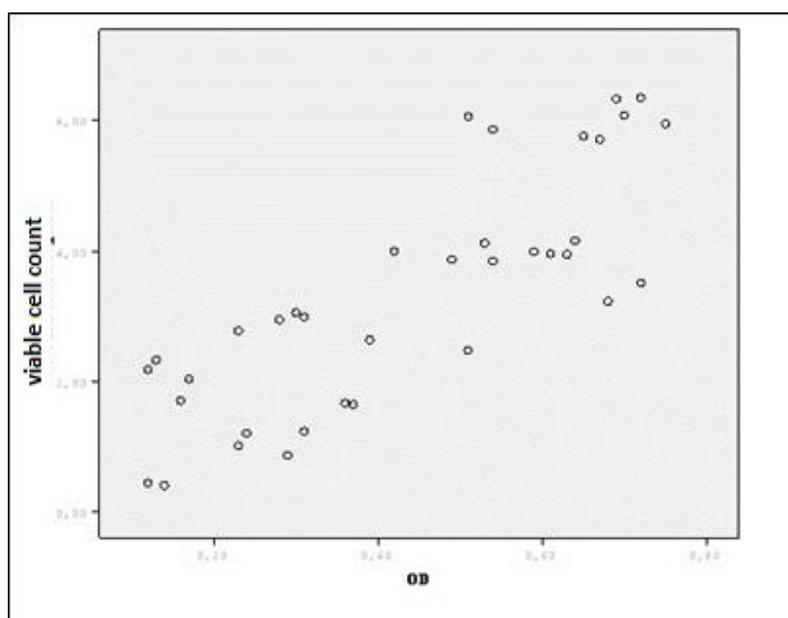


Figure 1. Relationship between biofilm inactivation tests based on viable cell count and OD.

3.4. Modelling *E. coli* Biofilm Elimination with Regression Analysis

Predictive modelling of pathogen bacteria during decontamination process gives useful information for the quantitative assessment of microbial risk, in addition to suggesting tools for comparing the importance of different inactivation methods [14]. It is not possible to predict the inactivation of foodborne pathogens with complete accuracy with mathematical models, but these models offer a possibility. The prediction of microbial inactivation with mathematical models helps process optimization regarding food safety [24,51].

In our study, prediction models of biofilm inactivation in terms of viable cell count and OD were developed with multiple linear regression analyses. In the multiple linear regression analysis, the classical method was used for 3 independent variables (X_1 =temperature, X_2 =time and X_3 =solution) and 1 dependent variable (Y =decrease in cell number or optical density) and the following regression equation model was used. This regression equation model made it possible to estimate the effect of sonication treatment at different conditions on *E. coli* biofilm inactivation. The developed models in the present research have advantages to predict the resistance of *E. coli* biofilm against sonication treatment under different conditions. The determination coefficient (R^2) was applied to judge how much the variability of the response variable can be influenced by the independent variables. Generally, R^2 values close to 1 represent the better fitting ability of prediction model [14]. Low values of Adj. R^2 (below 0.7) are indicators of non-adequacy of the models to explain the effect of the independent variables on the response [35]. According to Kavuncuoğlu et al. [22], the results of multiple linear regression ($R < 0.8$) demonstrated not a good agreement between the predicted and the experimental values. Regression model of the present study exhibited the goodness of fit of the regression equation with the R^2 and Adj. R^2 of 0.847 and 0.833, respectively. In summary, the present results of R^2 demonstrated that the model to predict the effect of sonication time, temperature and treatment solution on the inactivation of *E. coli* biofilm showed a very good fit.

The regression equation model (Eq. 2) explaining the biofilm inactivation rate (%) based on viable cell count is given below. Temperature, time and solution variables together exhibited a significant relationship with the biofilm inactivation rate ($R: 0.921$, $R^2: 0.847$, Adjusted $R^2: 0.833$). Independent variables including temperature, time and solution together explained 84% of the change in the biofilm inactivation rate. The decontamination efficiency of the independent variables exhibited different levels of statistical significance. According to the standardized regression coefficients, the relative importance order of the independent variables on the biofilm inactivation rate is solution ($\beta=0.569$), time ($\beta=0.555$) and temperature ($\beta=0.464$). Considering the significance tests

of the regression coefficients, it was seen that all independent variables (temperature, time and solution) had a significant effect ($p < 0.01$) on the inactivation rate. The data from viable cell count also indicated that biofilms of *E. coli* was more sensitive to the organic acids than other variables.

$$Y = -44.679 + 0.769X_1 + 7.642X_2 + 14.400X_3 \quad (2)$$

The regression equation model (Eq. 3) explaining the biofilm removal rate (%) based on optical density is given below. The temperature, time and solution variables exhibited a significant relationship (R: 0.898, R²: 0.806, Adj. R: 0.800) with the biofilm removal rate. Independent variables including temperature, time and solution together explain 80% of the change in the inactivation rate. According to standardized regression coefficients, the relative importance order of independent variables on the inactivation rate is time ($\beta=0.647$), temperature ($\beta=0.601$) and solution ($\beta=0.163$). Considering the significance tests of regression coefficients, it was seen that independent variables temperature and time had a significant effect on the biofilm removal rate at 99% confidence interval ($p < 0.01$) and solution at 95% confidence interval ($p < 0.05$ and solution). The data from OD measurement also indicated that biofilms of *E. coli* was more sensitive to treatment time than other variables.

$$Y = -30.478 + 0.961X_1 + 8.603X_2 + 3.970X_3 \quad (3)$$

The effectiveness of microbial inactivation methods depends on many factors such as the type of treatment, the physiology and type of the target microorganism, surface characteristics, treatment time, temperature, pH, concentration, etc. Damaging of food contacts surface and the presence of residues from disinfectants is undesirable in food industry. In this respect, combined inactivation techniques were applied against stress of single inactivation techniques such as high treatment time and temperature, high amount disinfectants, etc. [52]. However, numerous factors affecting biofilm inactivation requires mathematical models. Predictive modelling using sonication is a strategy to explain the inactivation of microorganisms according to previous researchers [34,35]. Previous works showed that good statistics adjustments was provide with models for microbial inactivation by sonication. But, to our literature review, until now, no research has modelled the elimination of *E. coli* biofilm by combined thermosonication and organic acids using multiple regression model. Regression models was used in various studies about *E. coli* biofilm inactivation on polystyrene surfaces or other food contact surfaces with various disinfectants (essential oils, chemical antimicrobial substances, ultrasound, thermal inactivation techniques etc) [4,24]. However, there are limited studies about prediction modelling of biofilm inactivation with thermosonication treatment. Newly developed models specific to inactivation method provide a better fit between the experimental and predicted data [22,23,53]. In a previous study, it was used linear and nonlinear regression models to predict *E. coli* inactivation and suggested the multiple linear regression model (R²=0.86) with its better prediction [51]. Similarly, in this study, prediction of *E. coli* biofilm inactivation has high accuracy with multiple regression model. The R² values of fitted models for biofilm inactivation in terms of viable cell count and OD were 0.84 and 0.80, respectively. This means that prediction modelling with viable cell count was better compared with OD. This study supported the hypothesis that regression modelling with live cell count is more reliable than that with OD [48,49].

In multiple linear regression analyses, residual values are also examined for the variability that the model function cannot explain. The residual value is used for the deviations between the observed value and the predicted value of the dependent variable. The normal P-P plot was used to determine the normality of the residual values. The closer the data in the graph is to the diagonal line, the closer the residual values are to a normal distribution. In other words, the P-P graph is used to see how well your data fits the normal distribution. If your data fits the normal distribution, your graph will follow the line perfectly. However, if your data does not fit the normal distribution, the line will fluctuate irregularly. This shows how much your data deviates from the normal distribution [21]. The scatter plots of observed values versus predicted values were visualized readily to show the suitability of the predictive models in Figures 2 and 3. Plots (Figure 2 ve 3) showing the probability of *E. coli* biofilm inactivation after single and combined sonication treatments were generated from the predictive equations (equation 2 and 3). For all models in this study, the p value was < 0.05 or < 0.01 and the

multiple correlation coefficient (R^2 , showing fitness of the model) was 0.84 and 0.80 showing that correlation with the predicted and observed values was good. The fitting curves of prediction models obtained in terms of viable cell count and OD showed similar reduction trends in Figures 2 and 3. According to data, prediction models obtained in terms of viable cell count was more suitable to describe the biofilm elimination under each treatment as represented R^2 (0.84).

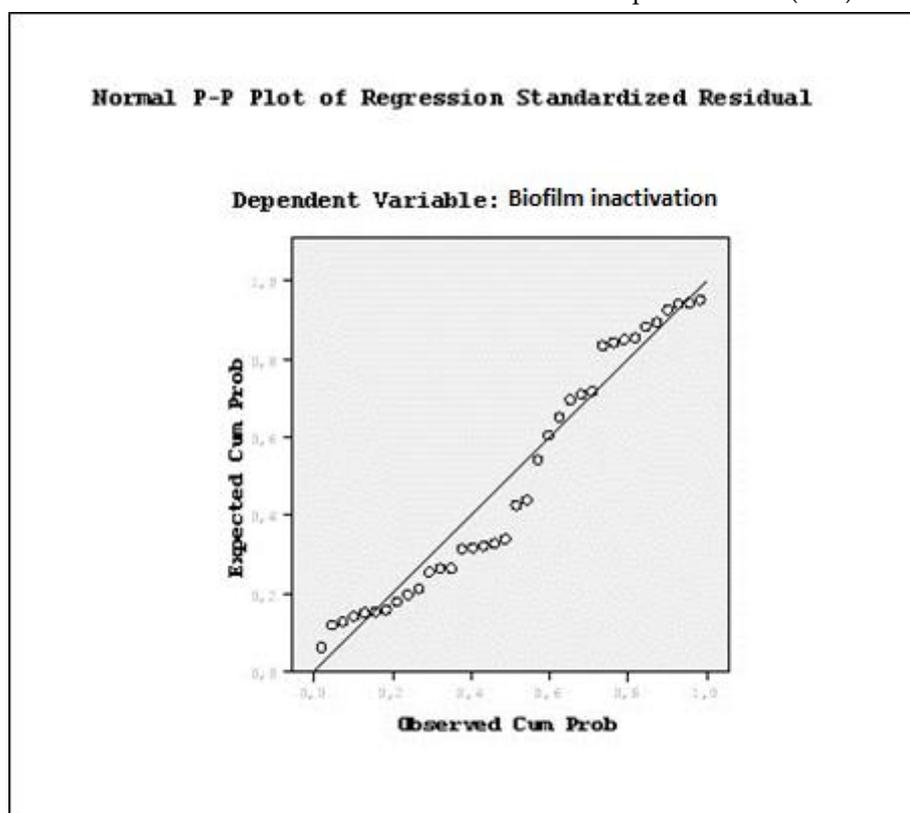


Figure 2. Scatter plot of observed and predicted values in terms of viable cell counts.

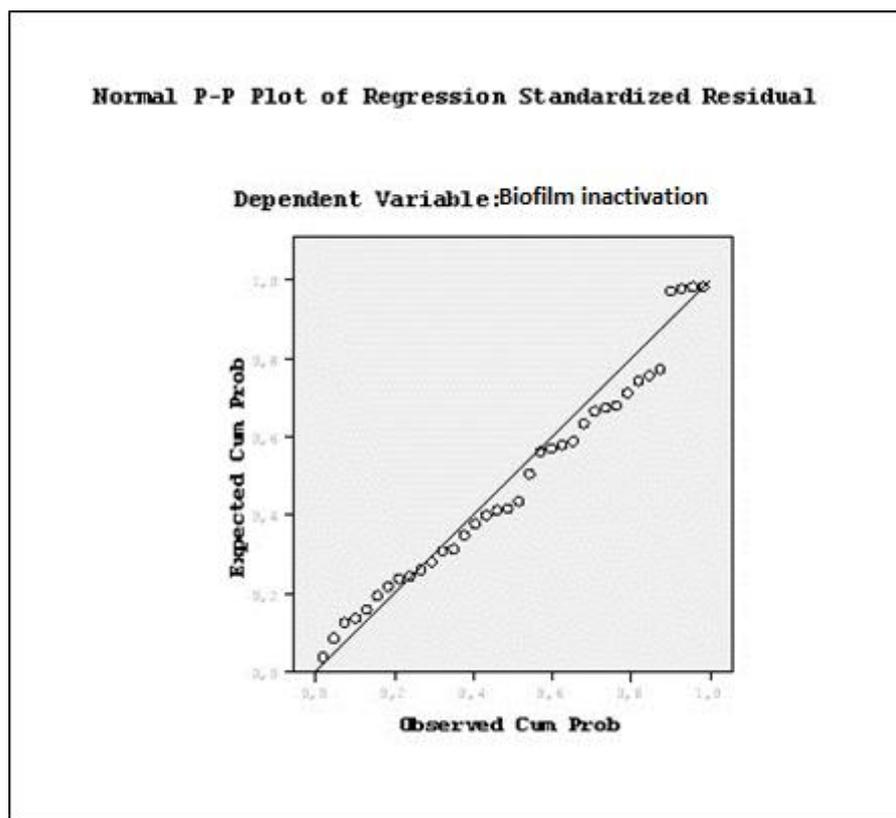


Figure 3. Scatter plot of observed and predicted values in terms of OD.

4. Conclusions

The present study indicated that combined treatment of sonication with organic acids significantly enhanced removal of *E. coli* biofilm on polystyrene surfaces compared with single thermosonication treatment. With the sonication process under different conditions, the number of *E. coli* biofilm live cells decreased by 0.43-6.21 log CFU/mL and the optical density (OD600) decreased by 0.13-0.72. A positive significant correlation was found between test methods based on viable cell count and optical density measurement. Combination treatment of organic acid and thermosonication at 50°C for 5 min was identified to be the most effective method against *E. coli* biofilm. However, *E. coli* biofilms were not completely removed from polystyrene surfaces after all treatments. This information is of special importance to improve the efficacy of inactivation of *E. coli* biofilm. Regression modelling of *E. coli* biofilm elimination gave an accurate estimation about microbial death. Scatter plot from data of viable cell count demonstrated closer relationship between the observed and predicted values than that of OD. The prediction models present potential for risk assessment planning and the selection of appropriate sonication treatment in food industry. Development of predictive models for *E. coli* biofilm inactivation on food contact surfaces under different sonication conditions contributes risk assessment through food chain from farm to fork. The more accurate the models, the more accurate our predictions, and this will expand their practical application.

References

1. Moreno, M.I.; Lomelía, M.L.; Novoa, M.G.A. Kinetics of biofilm formation by pathogenic and spoilage microorganisms under conditions that mimic the poultry, meat, and egg processing industries. *Int. J. Food Microbiol.* **2019**, *303*, 32-41, 2019.
2. Bang, J.; Hong, A.; Kim, H.; Beuchat, L.R.; Rhee, M.S.; Kim, Y.; Ryu, J.H. Inactivation of *Escherichia coli* O157:H7 in biofilm on food-contact surfaces by sequential treatments of aqueous chlorine dioxide and drying. *Int. J. Food Microbiol.* **2014**, *191*, 129-134.
3. Liu, X.; Yao, H.; Zhao, X.; Ge, C. Biofilm formation and control of foodborne pathogenic bacteria. *Molecules* **2023**, *28*, 2432, 1-19.

4. Zhao, L.; Poh, C.N.; Wu, J.; Zhao, X.; He, Y.; Yang, H. Effects of electrolysed water combined with ultrasound on inactivation kinetics and metabolite profiles of *Escherichia coli* biofilms on food contact surface. *Innov. Food Sci. Emerg.* **2022**, *76*(102917), 1-13.
5. Srey, S.; Park, S.Y.; Jahid, I.K.; Ha, S.D. Reduction effect of the selected chemical and physical treatments to reduce *L. monocytogenes* biofilms formed on lettuce and cabbage. *Food Res. Int.* **2014**, *62*, 484-49.
6. Sharma, S.; Jaiswal, S.; Duffy, B.; Jaiswal, A.K. Advances in emerging technologies for the decontamination of the food contact surfaces. *Food Res. Int.* **2022**, *151*, 110865, 1-12.
7. Yu, T.; Ma, M.; Sun, Y.; Xu, X.; Qiu, S.; Yin, J.; Chen, L. The effect of sublethal concentrations of benzalkonium chloride on the LuxS/AI-2 quorum sensing system, biofilm formation and motility of *Escherichia coli*. *Int. J. Food Microbiol.* **2021**, *353*, 109313, 1-7.
8. Unal Turhan, E.; Polat, S.; Erginkaya, Z.; Konuray, G. Investigation of synergistic antibacterial effect of organic acids and ultrasound against pathogen biofilms on lettuce. *Food Bioscience* **2022**, *47*, 1-8.
9. Lee, H.; Zhou, B.; Feng, H.; Martin, S.E. Effect of pH on inactivation of *Escherichia coli* K12 by sonication, manosonication, thermosonication, and manothermosonication. *J. Food Sci.* **2009**, *74*(4), 191-198.
10. Jose, J.F.B.S.; Andrade, N.J.; Ramos, A.M.; Vanetti, M.C.D.; Stringheta, P.C.; Chaves, J.B.P. Decontamination by ultrasound application in fresh fruits and vegetables. *Food Control* **2014**, *45*, 36-50.
11. Rani, M.; Sood, M.; Bandral, J.D.; Bhat, A.; Gupta, I. Thermosonication technology and its application in food industry. *Int. J. Chem. Stud.* **2020**, *8*(3), 922-928.
12. Abdulstar, A.R.; Altemimi, A.B.; Al-Hilphy, A.R. Exploring the power of thermosonication: A comprehensive review of its applications and impact in the food industry. *Foods* **2023**, *12*(1459), 1-22.
13. Urango, A.C.M.; Srieder, M.M.; Silva, E.K.; Meireles, M.A.A. Impact of thermosonication processing on food quality and safety: A review. *Food Bioprocess Technol.* **2022**, *15*, 1700-1728.
14. Esua, O.J.; Sun, D.W.; Ajani, C.K.; Cheng, J.H.; Keener, K.M. Modelling of inactivation kinetics of *Escherichia coli* and *Listeria monocytogenes* on grass carp treated by combining ultrasound with plasma functionalized buffer. *Ultrason. Sonochem.* **2022**, *88*, 106086, 1-11.
15. Carvalho, D.; Chitolina, G.Z.; Wilsmann, D.E.; Lucca, V.; Emery, B.D.; Borges, K.A.; Furian, T.Q.; Santos, L.R.; Moraes, H.L.S.; Nascimento, V.P. Development of predictive modeling for removal of multispecies biofilms of *Salmonella enteritidis*, *Escherichia coli*, and *Campylobacter jejuni* from poultry slaughterhouse surfaces. *Foods* **2024**, *13*, 1703, 1-12.
16. Avila, C.R.; Hascoet, A.S.; Guerrero-Navarro, A.E.; Rodríguez-Jerez, J.J. Establishment of incubation conditions to optimize the in vitro formation of mature *Listeria monocytogenes* biofilms on food-contact surfaces. *Food Control* **2018**, *92*, 240-248.
17. Melcon, C.R.; Peláez, F.R.; Fernández, C.G.; Calleja, C.A.; Capita, R. Susceptibility of *Listeria monocytogenes* planktonic cultures and biofilms to sodium hypochlorite and benzalkonium chloride. *Food Microbiol.* **2019**, *82*, 533-540.
18. Amrutha, B.; Sundar, K.; Shetty, P.H. Effect of organic acids on biofilm formation and quorum signaling of pathogens from fresh fruits and vegetables. *Microb. Pathog.* **2017**, *111*, 156-162.
19. Bang, H.J.; Park, S.Y.; Kim, S.E.; Rahaman, M.M.F.; Ha, S.D. Synergistic effects of combined ultrasound and peroxy acetic acid treatments against *Cronobacter sakazakii* biofilms on fresh cucumber. *LWT-Food Sci. Technol.* **2017**, *84*, 91-98.
20. Kim, S.Y.; Kang, D.H.; Kim, J.K.; Ha, Y.G.; Hwang, J.Y.; Kim, T.; Lee, S.H. Antimicrobial activity of plant extracts against *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on fresh lettuce. *J. Food Sci.* **2011**, *76*(1), 1-7.
21. Erol, H. SPSS Paket Programı ile İstatistiksel Veri Analizi, ISBN:978-605-397-060-6, Publisher: Nobel Kitabevi, Ankara, Türkiye, **2010**, 548p.
22. Kavuncuoğlu, H.; Kavuncuoğlu, E.; Karatas, S.M.; Benli, B.; Sagdic, O.; Yalcin, H. Prediction of the antimicrobial activity of walnut (*Juglans regia* L.) Kernel aqueous extracts using artificial neural network and multiple linear regression. *J. Microbiol. Methods* **2018**, *148*, 78-8.
23. Mustapha, A.T.; Zhou, C.; Amanor-Atiemoh, R.; Owusu-Fordjour, M.; Wahia, H., Fakayode, O.A.; Ma, H. Kinetic modeling of inactivation of natural microbiota and *Escherichia coli* on cherry tomato treated with fixed multi-frequency sonication. *Ultrason. Sonochem.* **2020**, *64*, 10503, 1-9.
24. Vidacs, A.; Kerekes, E.; Rajko, R.; Petkovits, T.; Alharbi, N.S.; Khaled, J.M.; Vagvölgyi, C.; Krisch, J. Optimization of essential oil-based natural disinfectants against *Listeria monocytogenes* and *Escherichia coli* biofilms formed on polypropylene surfaces. *J. Mol. Liq.* **2018**, *255*, 257-262.
25. Park Y.J.; Chen, J. Control of the biofilms formed by curli- and cellulose- expressing shiga toxin-producing *Escherichia coli* using treatments with organic acids and commercial sanitizers. *J. Food Prot.* **2015**, *78*(5), 990-995.
26. Wang, H.; Wang, X.; Yu, L.; Gao, F.; Jiang, Y.; Xu, X. Resistance of biofilm formation and formed-biofilm of *Escherichia coli* O157:H7 exposed to acid stress. *LWT-Food Sci. Technol.* **2020**, *118*(108787), 1-6.
27. Trang, P.N.; Ngoc, T.T.A.; Masuda, Y.; Hohjoh, K.; Miyamoto, T. Antimicrobial resistance and biofilm formation of *Escherichia coli* in a Vietnamese Pangasius fish processing facility. *Heliyon* **2023**, *9*(20727), 1-8.

28. Akbaş, M.Y.; Çağ, S. Use of organic acids for prevention and removal of bacillus subtilis biofilms on food contact surfaces. *Food Sci. Technol. Int.* **2016**, *22*(7), 587-597.
29. Nesse, L.L.; Sekse, C.; Berg, K.; Johannesen, K.C.S.; Solheim, H.; Vestby, L.K.; Urdahl, A.M. Potentially pathogenic *Escherichia coli* can form a biofilm under conditions relevant to the food production chain. *Appl. Environ. Microbiol.* **2014**, *80*(7), 2042-2049.
30. Kwak, T.Y.; Kim, N.H.; Rhee, M.S. Response surface methodology-based optimization of decontamination conditions for *Escherichia coli* O157:H7 and *Salmonella Typhimurium* on fresh-cut celery using thermoultrasound and calcium propionate. *Int. J. Food Microbiol.* **2011**, *150*, 128-135.
31. Zhao, X.; Zhen, Z.; Wang, X.; Guo, N. Synergy of a combination of nisin and citric acid against *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Addit. Contam. Part A* **2017**, *34* (12), 2058-2068.
32. Fan K.; Wu, J.; Chen, L. Ultrasound and its combined application in the improvement of microbial and physicochemical quality of fruits and vegetables: A review. *Ultrason. Sonochem.* **2021**, *80*(105838), 1-6.
33. Shao, L.; Dong, Y.; Chen, X.; Xu, X.; Wang, H. Modeling the elimination of mature biofilms formed by *Staphylococcus aureus* and *Salmonella* spp. Using combined ultrasound and disinfectants. *Ultrason. Sonochem.* **2020**, *69* 105269, 1-7.
34. Ahmet Görgüç, A.; Gençdağ, E.; Okuroğlu, F.; Yılmaz, F.M.; Bıyık, H.H.; Oztürk Kose, S.; Ersus, S. Single and combined decontamination effects of power-ultrasound, peroxyacetic acid and sodium chloride sanitizing treatments on *Escherichia coli*, *Bacillus cereus* and *Penicillium expansum* inoculated dried figs. *LWT-Food Sci. Technol.* **2021**, *140*, 110844, 1-8.
35. Freitas, L.L.; Prudêncio, C.V.; Peña, W.E.L.; Vanetti, M.C.D. Modeling of *Shigella flexneri* inactivation by combination of ultrasound, pH and nisin. *LWT-Food Sci. Technol.* **2019**, *109*, 40-46.
36. Yuk, H.G.; Yoo, M.Y.; Yoon, J.W.; Moon, K.D.; Marshall, D.L.; Oh, D.H. Effect of combined ozone and organic acid treatment for control of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on lettuce. *J. Food Sci.* **2006**, *71*(3), 83-87.
37. Stopforth, J.D.; Samelis, J.; Sofos, J.N.; Kendal, P.A.; Smith, G.C. Influence of organic acid concentration on survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in beef carcass wash water and on model equipment surfaces. *Food Microbiol.* **2003**, *20*, 651-660.
38. Ji, Q.Y.; Wang, W.; Yan, H.; Qu, H.; Liu, Y.; Qian, Y.; Gu, R. The effect of different organic acids and their combination on the cell barrier and biofilm of *Escherichia coli*. *Foods* **2023**, *12*(3011), 1-14.
39. Fındık, S.; Gündüz, G.; Gündüz, E. Direct sonication of acetic acid in aqueous solutions. *Ultrason. Sonochem.* **2006**, *13*, 203-207.
40. Lee, H.; Zhou, B.; Liang, W.; Feng, H.; Martin, S.E. Inactivation of *Escherichia coli* cells with sonication, manosonication, thermosonication, and manothermosonication: Microbial responses and kinetics modelling. *J. Food Eng.* **2009**, *93*, 354-364.
41. Turhan, E.U.; Polat, S. The removal of foodborne pathogen biofilms with the treatment of ultrasound and/or organic acid, *The Black Sea Journal of Sciences* **2022**, *12*(2), 905-915.
42. Costello, K.M.; Velliou E.; Gutierrez-Merino, J.; Smet, C.; El Kadri, H.; Van Impe, J. F.; Bussemaker, M. The effect of ultrasound treatment in combination with nisin on the inactivation of *Listeria innocua* and *Escherichia coli*. *Ultrason. Sonochem.* **2021**, *79*, 105776.
43. Braiek, O.B.; Smaoui, S. Chemistry, safety, and challenges of the use of organic acids and their derivative salts in meat preservation. *J. Food Qual.* **2021**, Volume 2021, Article ID 6653190,1-20.
44. Salleh-Mack, S.Z.; Roberts; J.S. Ultrasound pasteurization: The effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922. *Ultrason. Sonochem.* **2007**, *14*, 323-329.
45. Lee, H.; Kim, H.; Cadwallader, K.R.; Feng, H.; Martin, S.E. Sonication in combination with heat and low pressure as an alternative pasteurization treatment – Effect on *Escherichia coli* K12 inactivation and quality of apple cider. *Ultrason. Sonochem.* **2013**, *20*(4), 1131-1138.
46. Yu, H.; Liu, Y.; Li, L.; Guo, Y.H.; Xie, Y.F.; Cheng, Y.L.; Yao, W.R. Ultrasound involved emerging strategies for controlling foodborne microbial biofilms. *Trends Food Sci. Technol.* **2020**, *96*, 91-101.
47. Koda, S.; Miyamoto, M.; Toma, M.; Matsuoka, T.; Maebayashi, M. Inactivation of *Escherichia coli* and *Streptococcus mutans* by ultrasound at 500 kHz. *Ultrason. Sonochem.* **2009**, *16*, 655-659.
48. Loske, A.M.; Tello, E.M.; Vargas, S.; Rodriguez, R. *Escherichia coli* viability determination using dynamic light scattering: a comparison with standard methods, *Arch Microbiol* **2014**, 1-7.
49. Park, S.Y.; Kim, C.G. A comparative study of three different viability tests for chemically or thermally inactivated *Escherichia coli*. *Environ. Eng. Res.* **2018**, *23*(3), 282-287.
50. Macia, M.D.; Rojo-Molinero, E.; Oliver, A. Antimicrobial susceptibility testing in biofilm-growing bacteria. *Clin. Microbiol. Infect.* **2014**, *20*(10), 981-990.
51. Sheen, S.; Huang, C.Y.; Ramos, R.; Chien, S.Y.; Scullen, O.J.; Sommers, C. Lethality prediction for *Escherichia coli* O157:H7 and uropathogenic *E. coli* in ground chicken treated with high pressure processing and trans-cinnamaldehyde. *J. Food Sci.* **2018**, 1-10.

52. Parish, M.E.; Beuchat, L.R.; Suslow, T.V.; Harris, L.J.; Garret, E.H.; Farber, J.N.; Busta, F.F. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 161-173.
53. Ding, T.; Wang, J.; Forghani, F.; Ha, S.D.; Chung, M.S.; Bahk, G.J.; Hwang, I.G.; Abdallah, E.; Oh, D.H. Development of Predictive Models for the Growth of *Escherichia coli* O157:H7 on Cabbage in Korea. *J. Food Sci.* **2012**, *77*(5), 257-263.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.