Karadeniz Fen Bilimleri Dergisi, 12(2), 905-915, 2022. DOI: 10.31466/kfbd.1173998

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377

Araştırma Makalesi / Research Article

The removal of foodborne pathogen biofilms with the treatment of ultrasound and/or organic acid

Emel ÜNAL TURHAN[1*], Süleyman POLAT[2]

Abstract

The purpose of this study is to investigate the effect of ultrasound and/or organic acids against *Escherichia coli* and *Listeria monocytogenes* biofilms on microplates. In the inactivation trials, pathogen biofilms formed on microplates were exposed to 2% organic acid (acetic, citric, malic and lactic acid) and/or ultrasound for 10, 30 and 60 minutes. Biofilm detachment effect of treatment with US and/or organic acid was tested by microplate method based on measuring of optical density. In this study, the removal of foodborne pathogen biofilms varied depending on the treatment method (single or combined), the treatment time and the type of organic acid ($P<0.05$). The combined treatment with organic acid and ultrasound created additional biofilm detachment. 60 min ultrasound treatment with organic acids caused the highest removal of *E. coli* (77%) and *L. monocytogenes* (70%) biofilms. Lactic acid and malic acid among organic acids were the most effective against both of pathogen biofilms on microplates. With the increasing treatment time, the greater biofilm detachment was observed on microplates. The combination treatment of organic acid and ultrasound ensured safe and more effective decontamination against pathogen biofilms on microplates according to single treatment. These findings indicate that ultrasound treatment combined with organic acids can successfully be applied as an environmentally friendly biofilm detachment technique in food industry.

Keywords: Biofilm, *Escherichia coli*, *Listeria monocytogenes*, Organic acid, Ultrasound.

Ultrason ve/veya organik asit muamelesiyle gıda kaynaklı patojen biyofilmlerin uzaklaştırılması

Öz

Bu çalışmanın amacı, mikroplaklardaki *Escherichia coli* ve *Listeria monocytogenes* biyofilmlerine karşı ultrason ve/veya organik asitlerin etkisini araştırmaktır. İnaktivasyon denemelerinde, mikroplaklarda oluşturulan patojen biyofilmler 10, 30 ve 60 dakika boyunca %2 oranında organik asit (asetik asit, sitrik asit, malik asit ve laktik asit) ve/veya ultrasona maruz bırakılmıştır. Ultrason ve/veya organik asitle muamelenin biyofilm ayırma etkisi optik yoğunluk ölçümüne dayalı mikroplak yöntemiyle test edilmiştir. Bu çalışmada, gıda kaynaklı biyofilmlerin uzaklaştırılması muamele yöntemine (tek veya kombine), muamele süresine ve organik asit türüne bağlı olarak değişmiştir ($P<0.05$). Organik asit ve ultrason ile kombine muamele daha fazla biyofilm ayrılması yaratmıştır. 60 dakika organik asitli ultrason muamelesi en yüksek *E. coli* (%77) ve *L. monocytogenes* (%70) uzaklaştırmasına neden olmuştur. Organik asitler arasında laktik asit ve malik asit, mikroplaklar üzerindeki patojen biyofilmlerin her ikisine karşı da en etkilidir. Artan muamele süresiyle mikroplaklar üzerinde daha çok biyofilm ayrılması gözlenmiştir. Organik asit ve ultrason kombinasyon muamelesi, tekli muameleye göre mikroplaklardaki patojen biyofilmlere karşı daha güvenli ve etkili dekontaminasyon sağlamıştır. Bu bulgular organik asitlerle kombine edilen ultrason muamelesinin gıda endüstrisinde çevre dostu bir biyofilm ayırma tekniği olarak başarılı bir şekilde uygulanabileceğini gösterir.

Anahtar Kelimeler: Biyofilm, *Escherichia coli*, *Listeria monocytogenes*, Organik asit, Ultrason.

[1]Osmaniye Korkut Ata University, Department of Food Technology, Faculty of Kadirli Applied Sciences, Osmaniye, Turkey, emelunal@osmaniye.edu.tr
[2]Çukurova University, Department of Food Engineering, Faculty of Agriculture, Adana, Turkey, polats@cu.edu.tr

[1]https://orcid.org/0000-0002-0284-574X [2]https://orcid.org/0000-0002-2112-3136

[*]**Sorumlu Yazar/Corresponding Author** **Geliş/Received:** 12.09.2022 **Kabul/Accepted:** 05.11.2022

1. Introduction

Listeria monocytogenes and *Escherichia coli* are important foodborne pathogens responsible for various outbreaks. Especially, biofilms of these pathogens are seen as a challenge for food industry. Biofilm is defined as the community that microorganisms form by attaching to each other or to surface surrounded by self-produced extracellular polymeric substances which protect bacteria against adverse conditions (Rodriguez et al., 2010). *L. monocytogenes* and *E. coli* strains may attach to various biotic (carcasses, fruits, vegetables, meat and dairy products) and abiotic (stainless steel, teflon, glass, polystyrene, polypropilene, PVC) surfaces. The presence of biofilm in food processing plants or products may not only lead to food-borne outbreaks but also financial losses. Therefore food companies and researchers focused on effective and safety control methods for biofilm inactivation (Galie et al., 2018). Biofilm matrix provides increased resistance to bacteria and protects these bacteria against antimicrobial substances (Yuan et al., 2021). Various physical and chemical methods regarding biofilm detachment have been performed until now. Novel approach regarding biofilm detachment is environmentally friendly inactivation treatment combined with ultrasound. In particular, there is a recent trend towards organic acid treatment based ultrasound for disinfection of various surfaces (Jose et al., 2014a; Srey at al., 2014).

Organic acids are generally recognized as safe (GRAS). The main organic acids used in food industry for the aim of decontamination are lactic acid, acetic acid, citric acid, malic acid and peracetic acid. (Lapena et al., 2019; Lepaus et al., 2021; Meireles et al., 2016). Organic acids disrupt cell function as a result of the diffusion across microbial cell membranes (Cho and Ha, 2021). Although organic acids as environmentally friendly sanitizers are often applied in the food industry, limited penetration and diffusion of organic acids in the biofilm structure make them hard to inactivate the inner cells because of the presence of exopolysaccharide as a protective layer (Amrutha et al. 2017; Yuan et al., 2021). Considering that these issues, as a recent and safety biofilm detachment approach, organic acid treatment may be combined with US treatment in order to enhance penetration and diffusion of organic acids in the biofilm structure. Ultrasound technique has mostly been applied for the decontamination of food and food processing area surfaces. The cavitation effect from ultrasound processing are mainly responsible for microbial cell death. However, the other inactivation mechanism of ultrasound is related to production of reactive compounds as peroxide hydrogen. Ultrasound was applied alone and with combined other inactivation methods for the decontamination of various foods. There is an increasing interest in ultrasound since it also plays an important role as a green novel technology in the environment sustainability. On the other hand, further studies are required to use ultrasound technology for the aim of microbial inactivation in food industry (Bilek and Turantaş, 2013; Jose et al., 2014b).

Until now, studies have mostly been carried out in order to inactivate foodborne pathogens with the use of chemical disinfectants (chlorine solutions, ethanol, hydrogen peroxide etc.), but there are limited studies on pathogen biofilm inactivation and also the use of environmentally friendly decontamination methods (Jose et al., 2012; Lapena et al., 2016; Yuan et al., 2021). In particular, safe and effective inactivation methods have been suggested for the removing of biofilms that are more robust to antimicrobial substances. Therefore, researchers have recently focused on the combination treatment of ultrasound with organic acids as a hurdle approach for the purpose of biofilm detachment. The aim of this study is to remove *E. coli* and *L. monocytogenes* biofilms on microplates with ultrasound and/or organic acids.

2. Materials and Methods

2.1. Chemicals and Bacterial cultures

Various organic acids were used as sanitizers to detach pathogen biofilms in microplates. These organic acids are 2% (w/v) citric acid (Sigma-Aldrich, Austria), 2% (v/v) lactic acid (Sigma-Aldrich, Austria), 2% (w/v) malic acid (DL-malic acid 99.0%) and 2% (v/v) acetic acid (Merck-Germany). Stock cultures of *E. coli* and *L. monocytogenes* were stored frozen at -20 °C and were prepared from 700 µL overnight culture in Tryptic Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) supplemented with 300 µL 87% glycerol stock. Cells from stock were firstly streak plated on Tryptic Soy Agar (TSA; Difco) plate and grown for 24 hours at 37 °C. To use in the experiments related to biofilm formation and inactivation, one single colony was taken from the plate, transferred into 10 ml TSB and incubated overnight (18-22 hours) at 37 °C (Cui et al., 2018; Srey et al., 2014).

2.2. Biofilm formation on microtiter plates

Microplate method was used for the biofilm formation. 200 µl of TSB that contain 1% overnight culture of *L. monocytogenes* or *E. coli* were added to each well of a 96-well plate. Microplates were incubated for 24 hours at 37 ^{o}C. The medium was removed and the biofilm was washed three times with 250 µL of phosphate buffered saline (PBS). Subsequently biofilm cells were stained with 250 µL 0.1% crystal violet for 30 min and washed thrice with 250 µL water to remove unbound crystal violet. After drying, attached crystal violet was dissolved in 250 µL absolute ethanol and absorbance was measured at 595 nm (Elisa Microplate Reader, Rayto RT-6000) (Amrutha et al., 2017; Bang et al., 2017).

2.3. Biofilm inactivation with the treatment of US and organic acids

Overnight (18 h) grown cultures at 37 °C were used to inoculate (1%) 96-well polystyrene microtiter plates containing 200 µL TSB incubated at 37 °C for 24 hours. The medium was removed and the biofilm was washed once with 250 µL of PBS. Subsequently, 250 µL of treatment solutions (organic acid and PBS) were added to each well and these microplates were subjected to single and combined treatments for 10, 30 and 60 min. Thus, a total of three different inactivation trials were conducted: I. Single organic acid treatment, II. Single US treatment, and III. Combined treatment of US and organic acid. For single organic acid treatment (I), microplates with organic acid were stable placed on a flat surface to remain stable and left at 18 °C for 10, 30 and 60 min. Trials with ultrasound were performed in ultrasound bath (35 kHz frequency, a power of up to 380 W, Bandelin Sonorex, RK 1028 H-Germany). For single US treatment (II), PBS was used as a treatment solution. PBS alone has no inactivation effect and thus wells in microplates were filled with PBS. Microplates with PBS were meticulously placed on US bath and exposed to US at 18 °C for 10, 30 and 60 min. For combined treatment (III), microplates with organic acid (2%) were meticulously placed on US bath and exposed to US at 18 °C for 10, 30 and 60 min. After exposure, the biofilms were washed once with 250 µL PBS by pipetting rigorously. Subsequently biofilm cells were stained with 250 µL 0.1% crystal violet for 30 min and washed thrice with 250 µL water to remove unbound crystal violet (Figure 1). After drying, attached crystal violet was dissolved in 250 µL absolute ethanol and absorbance was measured at 595 nm (Elisa Microplate Reader, Rayto RT-6000) (Amrutha et al., 2017; Bang et al., 2017; Srey et al., 2014).

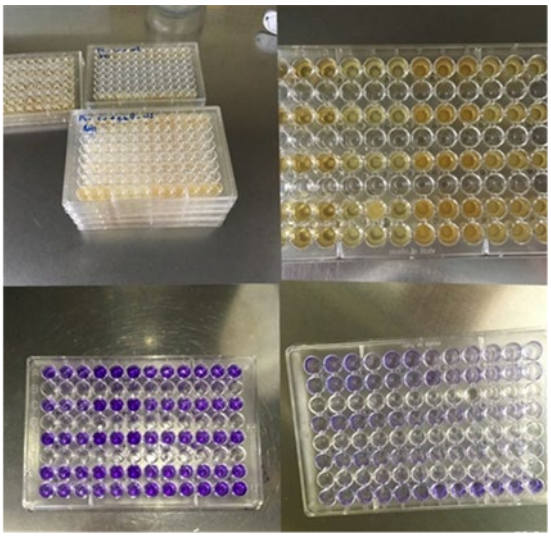

Figure 1. Some processing steps related with microplate method

2.4. Statistical analysis

All statistical analysis were conducted by SPSS version 20.0 (SPSS Inc., USA) statistical package. The values are given as mean ± standard deviation. The results were subjected to analysis of variance, and significant differences were determined by Duncan's multiple comparison tests ($P<0.05$).

3. Findings and Discussion

In this study, both of pathogen bacteria exhibited ability of biofilm formation on microplates. According to measurement of absorbance at 595 nm, optical density of *L. monocytogenes* and *E. coli* biofilms was 0.70 and 0.94, respectively. Then, the plates containing these biofilms were exposed to US or/and organic acid treatments for 10, 30 and 60 minute and, which caused detachment of pathogen biofilms in plates.

Table 1 shows results about the effect of treatment solution and time on *E. coli* biofilm detachment. The efficacy of *E. coli* biofilm detachment varied according to organic acid type and treatment time ($P<0.05$). *E. coli* biofilm detachment obtained with 10 min treatment was statistically different from that with 30 and 60 min treatment. The most effective organic acids on *E. coli* biofilm detachment were found to be citric acid, lactic acid and malic acid for 10, 30 and 60 min treatment, respectively. In the trial of the single organic acid treatment, the detachment effect of treatment solution on *E. coli* biofilm was significant at 10 and 30 min except at 60 min. Differently, in the trial of the combined treatment with US and organic acids, treatment solution produced significant detachment on *E. coli* biofilm at 10 and 60 min ($P<0.05$). In the combined treatment, organic acids causing the highest biofilm removal were found to be citric acid (OD595 = 0.312), lactic acid (OD595 = 0.251) and malic acid OD595 = 0.212) for 10, 30 and 60 min treatments, respectively. However, there are no significant difference between malic acid and lactic acid treatment for all treatment times. Additionally, the most effective detachment of *E. coli* biofilm was obtained on plates exposed to malic acid or lactic acid solution with 60 min US treatment.

Table 1. *E. coli* biofilm detachment in microplates

Treatment time (min)	Treatment solution (2%)	Absorbance (OD_{595}) after treatment without US	Absorbance (OD_{595}) after treatment with US
10	Acetic acid	$0.435 \pm 0.011_{b}^{A}$	$0.405 \pm 0.049_{a}^{AB}$
	Malic acid	$0.365 \pm 0.007_{c}^{CDE}$	$0.414 \pm 0.005_{a}^{CD}$
	Citric acid	$0.315 \pm 0.008_{d}^{BC}$	$0.312 \pm 0.065_{b}^{AB}$
	Lactic acid	$0.355 \pm 0.030_{c}^{BCD}$	$0.306 \pm 0.041_{b}^{CD}$
	PBS	$0.480 \pm 0.018_{a}^{A}$	$0.466 \pm 0.028_{a}^{A}$

30	Acetic acid	$0.374 \pm 0.019_{a}^{B}$	$0.316 \pm 0.150_{a}^{CD}$
	Malic acid	$0.303 \pm 0.017_{b}^{DE}$	$0.301 \pm 0.014_{a}^{CD}$
	Citric acid	$0.330 \pm 0.009_{ab}^{BCDE}$	$0.267 \pm 0.055_{a}^{DE}$
	Lactic acid	$0.294 \pm 0.035_{b}^{E}$	$0.251 \pm 0.049_{a}^{DE}$
	PBS	$0.370 \pm 0.054_{a}^{BC}$	$0.376 \pm 0.093_{a}^{BC}$
60	Acetic acid	$0.363 \pm 0.045_{a}^{BC}$	$0.263 \pm 0.009_{ab}^{DE}$
	Malic acid	$0.292 \pm 0.013_{a}^{E}$	$0.212 \pm 0.022_{c}^{E}$
	Citric acid	$0.328 \pm 0.040_{a}^{BCDE}$	$0.244 \pm 0.015_{abc}^{DE}$
	Lactic acid	$0.319 \pm 0.048_{a}^{BCDE}$	$0.217 \pm 0.030_{bc}^{E}$
	PBS	$0.362 \pm 0.035_{a}^{BC}$	$0.282 \pm 0.037_{a}^{DE}$

The results are shown as standard deviations ± average. ^{A,B,C,D,E} The different uppercase letters in the same column indicate a significant difference in the Duncan test. ^{a,b,c,d} Lowercase letters were used to indicate the difference between applications made in equal durations in the same column ($P<0.05$).

Table 2 gives results about the effect of US treatment on *E. coli* biofilm detachment. The data in Table 2 are the average results of 10, 30 and 60 minutes treatments. The detachment effect of ultrasound was found significant for samples treated with acetic acid, citric acid and lactic acid ($P<0.05$). As seen from the present results, the detachment effect of ultrasound was insignificant ($P>0.05$) for samples treated with malic acid and PBS. Ultrasound processing showed its antibiofilm effect most with lactic acid solution (OD595 = 0.26). On the other hand, the lowest biofilm detachment (OD595 = 0.37) effect of US was observed in microplates treated with PBS solution. According to our results, ultrasound treatment created additional inactivation effect against *E. coli* biofilms on plates. These findings indicated that combined treatment was more effective for the removal of biofilm.

Table 2. The effect of ultrasound on *E. coli* biofilm detachment

Treatment solution (2%)	**Absorbance (OD_{595}) after treatment without US**	**Absorbance (OD_{595}) after treatment with US**
PBS	0.40 ± 0.07^{a}	0.37 ± 0.09^{a}
Acetic acid*	0.39 ± 0.04^{a}	0.33 ± 0.07^{ab}
Malic acid	0.32 ± 0.02^{b}	0.31 ± 0.09^{b}
Citric acid*	0.32 ± 0.04^{b}	0.27 ± 0.05^{ab}
Lactic acid*	0.32 ± 0.04^{b}	0.26 ± 0.05^{b}

The results are shown as standard deviations ± average. ^{a,b} The different lowercase letters in the same column indicate a significant difference in the Duncan test. * indicate a significant difference according to T-test ($P<0.05$).

Table 3 presents results about the effect of treatment solution and time on *L. monocytogenes* biofilm detachment. *E. coli* biofilm detachment changed depending on organic acid type and treatment time. The effect of organic acid type on *L. monocytogenes* biofilm detachment was insignificant ($P>0.05$) except 30 min single organic acid treatment ($P<0.05$). In the trial of 30 min single organic acid treatment, citric acid among organic acids achieved the highest L. monocytogenes biofilm detachment (OD595 = 0.286). Similar to our results about *E. coli* biofilm detachment, the

effect of treatment time on *L. monocytogenes* biofilm detachment was significant (P<0.05) for the both trials (single organic acid treatment and combined treatment). With the increase of treatment time, the removal of *L. monocytogenes* biofilm increased. The highest *L. monocytogenes* biofilm detachment was observed with 60 min combined treatment of US and malic acid (OD595 = 0.194) or lactic acid (OD595 = 0.205).

Table 3. *L. monocytogenes* biofilm detachment in microplates

Treatment time (min)	Treatment solution (2%)	Absorbance (OD_{595}) after treatment without US	Absorbance (OD_{595}) after treatment with US
10	Acetic acid	$0.411 \pm 0.155_a^{AB}$	$0.257 \pm 0.042_a^{BC}$
	Malic acid	$0.349 \pm 0.013_a^{BCD}$	$0.220 \pm 0.005_a^{CDE}$
	Citric acid	$0.323 \pm 0.036_a^{BCD}$	$0.243 \pm 0.033_a^{CD}$
	Lactic acid	$0.358 \pm 0.037_a^{ABCD}$	$0.298 \pm 0.038_a^{B}$
	PBS	$0.446 \pm 0.046_a^{A}$	$0.342 \pm 0.042_a^{A}$
30	Acetic acid	$0.349 \pm 0.003_b^{BCD}$	$0.208 \pm 0.006_b^{DE}$
	Malic acid	$0.330 \pm 0.033_{bc}^{BCD}$	$0.214 \pm 0.006_b^{CDE}$
	Citric acid	$0.286 \pm 0.035_c^{D}$	$0.215 \pm 0.039_b^{CDE}$
	Lactic acid	$0.331 \pm 0.009_{bc}^{BCD}$	$0.227 \pm 0.004_b^{CDE}$
	PBS	$0.402 \pm 0.036_a^{ABC}$	$0.294 \pm 0.003_a^{B}$
60	Acetic acid	$0.308 \pm 0.030_a^{CD}$	$0.204 \pm 0.021_b^{DE}$
	Malic acid	$0.348 \pm 0.017_a^{BCD}$	$0.194 \pm 0.013_b^{E}$
	Citric acid	$0.302 \pm 0.024_a^{D}$	$0.194 \pm 0.024_b^{E}$
	Lactic acid	$0.328 \pm 0.038_a^{BCD}$	$0.205 \pm 0.009_b^{DE}$
	PBS	$0.363 \pm 0.013_a^{ABCD}$	$0.242 \pm 0.003_a^{CD}$

The results are shown as standard deviations ± average. ^{A,B,C,D,E} The different uppercase letters in the same column indicate a significant difference in the Duncan test. ^{a,b,c} Lowercase letters were used to indicate the difference between applications made in equal durations in the same column (P<0.05).

Table 4 presents results about the effect of US treatment on *L. monocytogenes* biofilm detachment. The data in Table 4 are the average results of 10, 30 and 60 minutes treatments. The biofilm removal effect of ultrasound was found to be significant (P<0.05) in samples treated with all organic acid solutions except PBS solution. The combination treatments of organic acid and US caused higher *L. monocytogenes* biofilm detachment compared with single organic acid treatment. While *L. monocytogenes* biofilm detachment effect of ultrasound was obtained most with malic acid solution (OD595 = 0.209), its lowest antibiofilm effect was observed with PBS solution (OD595 = 0.292).

Table 4. The effect of ultrasound on *L. monocytogenes* biofilm detachment

Treatment solution (2%)	Absorbance (OD_{595}) after treatment without US	Absorbance (OD_{595}) after treatment with US
PBS	0.404 ± 0.047^a	0.292 ± 0.048^a

Acetic acid *	0.356±0.091[ab]	0.223±0.035[b]
Malic acid *	0.342 ±0.032[b]	0.209±0.014[b]
Citric acid *	0.304±0.032[b]	0.217±0.035[b]
Lactic acid *	0.339±0.030[b]	0.243±0.046[b]

The results are shown as standard deviations ± average. [a,b] The different lowercase letters in the same column indicate a significant difference in the Duncan test. * indicate a significant difference according to T-test ($P<0.05$).

L. monocytogenes and *E. coli* are important foodborne pathogens capable of biofilm formation on food and food contact surfaces (Aryal and Muriana, 2019). Similarly, the present study confirms that *L. monocytogenes* and *E. coli* strains are adhered to microplates and form biofilm according to the results of optical density (OD595 = 0.70 and 0.94, respectively). After the inactivation treatments, reduction in OD595 values of *L. monocytogenes* and *E. coli* biofilms treated with US and organic acid was 0.50 and 0.73 unit, respectively. As seen from the present results, *E. coli* biofilm showed higher sensitivity to detachment treatments than *L. monocytogenes* biofilm. Gram-negative and Gram-positive bacteria such as *E. coli* and *L. monocytogenes* may exhibit different resistance mechanism. Gram-negative bacteria are more susceptible to low pH than Gram-positive bacteria (Ban et al., 2012. Ban et al. (2012) reported that lactic acid produced significant reductions of *L. monocytogenes* and *E. coli* biofilms on polyvinylchloride (PVC) and stainless steel coupons. In another study, after peracetic acid exposure to microplates containing *L. monocytogenes* biofilm, *L. monocytogenes* biofilm reduction was observed in microplates (Korany et al., 2018). In this study, single organic acid treatment was compared with the combination treatment of US and organic acid and both of treatment caused biofilm detachment for 60 min. Percentages of *E. coli* biofilm detachment were obtained maximum 69% with single organic acid treatment and 77% with the combination treatment. Percentages of *L. monocytogenes* biofilm detachment with single and the combination treatment were found to be maximum 58% and 70%, respectively. In the study of Borges et al. (2012), percentages of pathogen biofilm detachment from exposure to ferulic and gallic acids were reported between 20% and 40% for *E. coli* strains and 5 and 10% for *L. monocytogenes* strains on microplates. Combined treatments with US were applied for more effective or enhanced inactivation against foodborne pathogen biofilms (Bang et al., 2017, Francisco et al., 2017, Jose et al., 2014b, Zho et al., 2017). Ultrasound treatment facilitated penetration of organic acid into the biofilm matrix (Alenyorege at al., 2019). The penetration of the organic acids through microbial cell membrane is provided by the intense pressure gradients and cavitation phenomena from US (Lapena et al., 2019). This mechanism indicates that the application of US in order to remove pathogen biofilm is more essential compared with planktonic cell. Therefore, recent studies have been conducted in order to inactivate pathogen biofilms with the combination treatment of US and various antimicrobial agents such as organic acids, chlorine compounds, essential oils etc. (Torlak et al, 2013). As known from the present study, single ultrasound treatment (10 min) caused 51% biofilm detachment for both

strains (*E. coli* and *L. monocytogenes*). In a study about single ultrasound treatment, 5 min US caused approximately 40% detachment of *L. monocytogenes* biofilm on plastic food contact surface (Torlak et al., 2021). All these data confirms that the pathogen biofilm detachment varies depending on the treatment time, the type of organic acid and species of target microorganism (Lapena et al., 2019).

4. Conclusions and Recommendations

As a result of this study, the removal of biofilm varied depending on the type of organic acid, treatment time and inactivation method. Ultrasound created additional inactivation action on *L. monocytogenes* and *E. coli* biofilms. The highest *L. monocytogenes* and *E. coli* biofilm detachment was obtained with the combined treatment of US and malic acid or lactic acid for 60 min. However, the next studies should be conducted about biofilm inactivation on various food surfaces.

Acknowledgements

This research was supported by the Scientific and Technical Research Council of Turkey (TUBITAK, Project Number: 118O731) and Research Council of Osmaniye Korkut Ata University (Project Number: OKÜBAP-2019-PT2-002).

Authors' Contributions

Emel ÜNAL TURHAN: Designing the experiment, Performing the experiment, Collecting data, Conceptualization, Methodology, Investigation, Collecting data, Evaluating the data, Writing initial and original draft, Reviewing and editing the manuscript. Suleyman POLAT: Designing the experiment, Performing the experiment, Collecting data, Evaluating the data, Software and validation, Methodology, Reviewing and editing the manuscript.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

References

Alenyorege, E. A., Ma, H., Ayim, I., Lu, F. and Zhou, C. (2019). Efficacy of sweep ultrasound on natural microbiota reduction and quality preservation of Chinese cabbage during storage. *Ultrason Sonochemisty*, 59, 1-10.

Amrutha, B., Sundar, K. and Shetty, P. H. (2017). Effect of organic acids on biofilm formation and quorum signaling of pathogens from fresh fruits and vegetables. *Microbial Pathogenesis*, 111, 156-162.

Aryal, M. and Muriana P. M. (2019). Efficacy of commercial sanitizers used in food processing facilities for inactivation of *Listeria monocytogenes, E. coli* O157:H7, and *Salmonella* biofilms. *Foods*, 8, 1-14.

Ban, G. H., Park, S. H., Kim, S. O., Ryu, S. and Kang, D. H. (2012). Synergistic effect of steam and lactic acid against *Escherichia coli* O157:H7, *Salmonella Typhimurium*, and Listeria monocytogenes biofilms on polyvinyl chloride and stainless steel. *International Journal of Food Microbiology*, 157, 218-223.

Bang, H. J., Park, S. Y., Kim, S. E., Rahaman, M. M. F. and Ha, S. D. (2017). Synergistic effects of combined ultrasound and peroxy acetic acid treatments against *Cronobacter sakazakii* biofilms on fresh cucumber. *LWT-Food Science and Technology*, 84, 91-98.

Bilek, S. E. and Turantaş, F. (2013). Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review. *International Journal of Food Microbiology*, 166, 155-162.

Borges, A., Saavedra, M. J. and Simoes, M. (2012). The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. *Biofouling*, 28, 755–767.

Cho, G. L. and Ha, J. W. (2021). Synergistic effect of citric acid and xenon light for inactivating foodborne pathogens on spinach leaves. *Food Research International*, 142, 1-9.

Cui, H., Bai, M., Yuan, L., Surendhiran, D. and Lin, L. (2018). Sequential effect of phages and cold nitrogen plasma against *Escherichia coli O157:H7* biofilms on different vegetables. *International Journal of Food Microbiology*, 268, 1-9.

Francisco, C. A. I., Naves, E. A. A., Ferreira, D. C., Do, Rosario, D. K. A., Cunha, M. F. et al (2017). Synergistic effect of sodium hypochlorite and ultrasound bath in the decontamination of fresh arugulas. *Journal of Food Safety*, 38, 1-7.

Galie, S., Gutiérrez, C. G., Miguélez, E. M., Villar C. J. and Lombó (2018). Biofilms in the food industry: Health aspects and control methods. *Frontiers in Microbiology*, 9, 1-18.

Jose, J. F. B., Medeiros, H. S., Bernardes, P. C. and Andrade, N. J. (2014a). Removal of *Salmonella enterica Enteritidis* and *Escherichia coli* from green peppers and melons by ultrasound and organic acids. *International Journal of Food Microbiology*, 190, 9-13.

Jose, J. F. B. S., Andrade, N. J., Ramos, A. M., Vanetti, M. C. D. and Stringheta, P. C. (2014b). Decontamination by ultrasound application in fresh fruits and vegetables. *Food Control*, 45, 36-50.

Jose, J. F. B. S. and Vanetti, M. C. D. (2012). Effect of ultrasound and commercial sanitizers in removing natural contaminants and *Salmonella enterica Typhimurium* on cherry tomatoes. *Food Control*, 24, 95-99.

Korany, A. M., Hua, Z., Green, T, Hanrahan, I., El-Shinawy, S. H. et al (2018). Efficacy of ozonated water, chlorine, chlorine dioxide, quaternary ammonium compounds and peroxy acetic acid against *Listeria monocytogenes* biofilm on polystyrene surfaces. *Frontiers in Microbiology*, 9, 1-10.

Lapeña, I. N., Lafarga, T., Viñas, I., et al (2019). Ultrasound processing alone or in combination with other chemical or physical treatments as a safety and quality preservation strategy of fresh and processed fruits and vegetables: A review. *Food Bioprocess Technology*, 12, 1452-1471.

Lepaus, B. M., Rocha, J. S. and Jose, J. F. B. S. (2021). Organic acids and hydrogen peroxide can replace chlorinated compounds as sanitizers on strawberries, cucumbers and rocket leaves. *Food Science and Technology*, 40, 242-249.

Meireles, A., Giaouris, E. and Simõesal, M. (2016). Alternative disinfection methods to chlorine for use in the fresh-cut industry. *Food Research International*, 82, 71-85.

Rodriguez, N., Salgado, J. M., Cortes, S. and Dominguez, J. M. (2010). Alternatives for biosurfactants and bacteriocins extraction from *Lactococcus lactis* cultures produced under different pH conditions. *Letters in Applied Microbiology*, 51, 226-233.

Srey, S., Park, S. Y. and Jahid, I. K. (2014). Reduction effect of the selected chemical and physical treatments to reduce *L. monocytogenes* biofilms formed on lettuce and cabbage. *Food Research International*, 62,484-49.

Torlak, E. and Sert, D. (2013). Combined effect of benzalkonium chloride and ultrasound against *Listeria monocytogenes* biofilm on plastic surface. *Letters in Applied Microbiology*, 57, 220-226.

Yuan, L., Sadiq, F. A., Wang, N., Yang, Z. and He, G. (2021). Recent advances in understanding the control of disinfectant-resistant biofilms by hurdle technology in the food industry. *Critical Reviews in Food Science and Nutrition*, 61, 3876-3891.

Zhao, X., Zhen, Z., Wang, X. and Guo, N. (2017). Synergy of a combination of nisin and citric acid against *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Additives & Contaminants: Part A*, 34, 2058-2068.