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Antiviral Efficacy of Dielectric Barrier Discharge Plasma against Hepatitis A Virus in Fresh Oyster Using PMA/RT-qPCR

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Abstract: The hepatitis A virus (HAV), which can be a threat to humans, can be found in oysters. Oysters need sterilization technology that does not cause quality changes because they are eaten raw. Therefore, in this study, antiviral effects and quality changes were conducted using dielectric barrier discharge plasma (DBDP). Total titers of non-PMA- and PMA-treated suspensions of HAV were reduced to 0.20–1.05 and 0.35–1.49 $\log_{10}$ copies/μL, respectively, after DBDP 10–60 min treatment. HAV titers on oysters were reduced to 0.20–1.11 and 0.33–1.33 $\log_{10}$ copies/μL, respectively, after identical an DBDP treatment of the non-PMA- and PMA-treated oysters. The HAV titers were revealed as 0.295 (=[0.25 for 30 min + 0.34 for 60 min]/2) and 0.22 (for 60 min) $\log_{10}$-reduction in PMA-treated suspensions and oysters, respectively, compared with the non-PMA-treated suspensions and oysters. The D_1 of DBDP in PMA-treated suspensions and oysters were 39.99 and 46.73 min, respectively. There was no difference in the pH of oysters after DBDP treatment compared to the non-treated control. There were some changes in the hardness of the DBDP-treated oysters, which was due to oyster individual differences rather than the DBDP treatment. In conclusion, DBDP treatment did not affect oyster quality and PMA was able to distinguish HAV infectivity.

Keywords: hepatitis A virus; suspension; oyster; DBD plasma; antivirus efficacy; quality

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1. Introduction

The hepatitis A virus (HAV) is a single-stranded RNA virus without an envelope that belongs to the family *Picornaviridae* [1]. HAV infection is mainly caused by exposure to contaminated food or water. Around the word, 100 million peopled are infected with HAV every year, and the resulting death toll is about 15,000–30,000 [2]. HAV is resistant to many environmental factors [3], and several studies have reported that HAV can survive for a long time outside the host and underwater, and remain contagious for at least 9 months [4]. It is also found in various foods such as shellfish [5,6], dairy products [7], vegetables and fruits [8,9], and food-borne HAV can infect humans through oral intake.

HAV infection can occur through the intake various foods and also through marine foods [10]. Since 1962, infections of shellfish have been regularly reported [11], and the outbreak areas are diverse, including Korea [12], Japan [13], China [14], Spain [15], and Australia [16]. Oyster (*Crassostrea gigas*) is a nutritious food with low fat content and large amounts of high-quality protein, taurine, vitamins, iron, and zinc [17,18]. However, oyster farms are adjacent to land, so they can be easily contaminated by waste and wastewater coming down from the land [19]. Additionally, Bivalvia, such as oysters and mussels, consume floating plankton through filterfeeding; therefore, harmful microbiological can easily accumulate in digestive tissues [20]. Oysters in particular are considered primary

vectors of HAV [21]. Therefore, to ensure sanitary safety, specific procedures are required due to the culinary features of oysters and the high intake rate of raw oysters.

Heat sterilization, a traditionally used sterilization technique, is still frequently used in the food industry [22]. One of the greatest benefits of heat sterilization is that it allows the production of foods with extremely long shelf lives. Although it is an effective method for eliminating germs such as bacteria and viruses, it can alter food quality [23]. Patel et al. [24] reported that heat sterilization changed the color and vitamin C in the content of food, and Hu et al. [25] also reported that the heat sterilization of apple juice changed its quality and functional aspects, as opposed to non-thermal sterilization. Recently, non-thermal sterilization technology using plasma, which can overcome the disadvantage of heat sterilization, has been in the spotlight. Plasma is defined as the fourth state of matter and includes active species such as electrons, cations, anions, free radicals, and ultraviolet photons [26]. These active species diffuse through the cell membrane, react with lipids and proteins in the cell membrane and intracellular DNA, and damage the cells [27,28]. One such method, atmospheric dielectric barrier discharge plasma (DBDP), which is produced under atmospheric pressure at room temperature, as opposed to being produced in a vacuum, and can be treated at temperatures below 35 °C, is gaining attention as a new method of food sterilization because it is a non-thermal method that does not alter the protein content or the quality and functional properties of food.

Therefore, our study investigated the anti-HAV effect of DBDP treatment in seafood. In addition, real-time polymerase chain reaction (RT-qPCR) was used for a quantitative analysis of HAV, and propidium monoazide (PMA) was used to detect viable viruses.

2. Materials and Methods

2.1. Preparation of HAV

HAV was purchased from a Korean Bank for Pathogenic Viruses (KBPV, Seoul, Republic of Korea) and delivered in a frozen state with an ice pack. The LLC-MK2 cell line was cultured for 5–8 days under 5% CO_2 conditions of 37 °C in a 2% phosphate-buffered saline Dulbecco's modified Eagle's medium (DMEM). The viral cell line was made in a stock containing a 2% PBS (factor 8.0×10^7 pfu/mL; pH 7.4).

2.2. HAV Inoculation of Suspension and Oysters

A suspension was prepared using a phosphate-buffered saline (PBS) solution. HAV 20 μL and PBS 80 μL were mixed when making suspension. It was then placed in a clean bench (CHC Lab Co., Ltd., Dajeon, Republic of Korea) for 1 h.

The oysters (*Crassostrea gigas*) used in this experiment came from an oyster farm (S Company in Geoje, Gyeongsangnam-do, Republic of Korea). The size of the oysters used in this experiment was 25–35 mm, and for freshness, they were washed with clean water immediately after receiving them, and the shells were removed. The midguts of the oysters were collected with sterilized tweezers and scissors, and homogenized using a homogenizer (Daihan Scientific Co., Wonju, Republic of Korea). Thereafter, 3 g per sample was divided into Petri dishes (30 × 30, L × W mm) and analyzed. Each divided sample was inoculated with 20 μL of HAV and placed in a clean bench (CHC Lab Co., Ltd., Daejeon, Republic of Korea) for 1 h.

2.3. Atmospheric DBDP Treatment

For atmospheric DBDP treatment, a DBDP device (μ-DBD Surface Plasma Generator, Model; Micro DBD Plasma) was used in this study. The method of using DBDP was demonstrated by Ryu et al. [29]. To generated DBDP, nitrogen gas supplied to a polylactic acid (PLA; Ultimaker, Utrecht, The Netherlands) cover at a flow of 1.5 L/m (liter per min), and plasma was generated on the rear glass surface between the glass and the mesh. As the dielectric, circular soda glass (diameter: 80 mm, thickness: 18 mm) was used. Circular soda glass (diameter: 80 mm, thickness: 18 mm) was used as a dielectric. In order to generate plasma, a mesh consisting of an upper silver electrode and a lower stainless steel

was attached to the back of the glass and used as a counter electrode. The frequency was 43 kHz, the voltage was measured to be about 1 kV, the peak discharge current of the cycle was 7.7 μJ, and energy per second was measured to be 0.12 J/s. During the experiment, the distance between the plasma device and the sample was maintained at 3 mm. Samples were treated with DBDP for 5, 10, 20, 30, and 60 min.

2.4. Propidium Monoazide Staining

Propidium monoazide (Biotium, Hayward, CA, USA) was used to detect infectious HAV. The method of using PMA dye was performed by Jung et al. [30]. First, PMA 7 μM, which is 1% of the capacity, was mixed with the sample solution (700 μL) that went through the pretreatment process. It was then placed in a dark room at room temperature for 10 min to allow the dye of the mixture to penetrate the cells, and the front and rear surfaces were exposed to 460 nm wavelengths of 40 W LED lights (Dynebio, Seongnam, Republic of Korea) for 10 min to activate the light. To confirm the effectiveness of the PMA treatment using RT-qPCR, the control was not treated with PMA.

2.5. RNA Extraction of HAV

The RNA extraction of HAV was isolated and purified using the QIAamp virus RNA mini kit (Qiagen, Hilden, Germany). Proteinase K was used according to the ISO 1f5216-1:2017 method. Proteinase K (Sigma, St. Louis, MO, USA) was added to the samples, incubated in a shaker at 37 °C for 1 h, and then deactivated in a 60 °C water bath for 15 min. Then, the sample was centrifuged at 4695× *g*, for 10 min at 4 °C (SUPRA22K; Hanil Science Industrial Co., Gimpo, Republic of Korea), and a clear supernatant containing viral RNA (approximately 3 mL) was collected in a sterilized conical tube.

2.6. Quantitative Analysis of HAV Using RT-qPCR

For a quantitative analysis of HAV, the RNeasy mini-kit (Qiagen, Hilden, Germany) was used. The mixture used for HAV gene amplification was prepared using RNase-free water, 5× RT-PCR buffer, 10 mM dNTP, 10 μM primer, 10 μM probe, enzyme mix (5 units/μL), and suspension (5 units/μL). The total volume used in the experiment was 25 μL, including 5 μL of RNA, and 20 μL of mixture, and real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed in TP800-Thermal Cycler Dice Real-Time System (Takara). Primers and probes were prepared for the overlapping regions of HAV to increase sensitivity and specificity. The sequences of the primers and probes are shown in Table 1.

Table 1. Sequence of primers and probe for RT-qPCR to quantitate HAV.

Type	Component	Sequence (5′→3′)
Primer	Forward	GCG GGG GAT ATT GGT GAG
	Reverse	CAA TGC ATC CAC TGG ATG AGA
Probe	HAV prove	TTA AGA CAA AAA CCA TTC AAC GCC GGA G

2.7. Determination of Decimal Reduction Times (D-Values) through First-Order Kinetic Model

The D-value is the treatment time required for a reduction of 1 log copies/μL (>90%). The formula for calculating the D-value and its explanation is as follows:

$$\log \frac{N_0}{N} = \frac{k}{2.303} \cdot t$$

N_0 = initial HAV titer (log copies/μL); N = HAV titer after DBDP treatment (log copies/μL); t = DBDP treatment time (min); k = reduction rate constant.

2.8. pH Value and Hardness Measurement

The pH measurement of the oysters was demonstrated by the Choi et al. [31] method. To measure the pH, 5 g of each oyster sample and 45 mL of diluted water were mixed and stirred for 5 min. The pH was measured three times using a pH meter (Orion Star A211, Thermo Scientific, Waltham, MI, USA).

The hardness measurements were performed using a CT3 texture analyzer (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) after the oysters were treated with DBDP. 'Hardness' was measured in the texture profile analysis (TPA) mode. The measurement conditions were: probe, stainless steel probe-type TA18 12.7 mm; test speed, 1.0 mm/s; force threshold, 20 g; distance threshold, 0.50 mm; compression limit, 50% deformation. The results were recorded after three repeated measurements within an error range of ±1 g using the same size and same part of the oyster.

2.9. Statistical Analysis for Significant Difference Verification

Statistical analysis was conducted to confirm a significant difference between the reduction (log copies/μL) of HAV titer, D-value, and the quality (pH, hardness) according to the DBDP treatment time. The statistical analysis results were calculated based on the value of repeating the experiment three times per sample and expressed as mean ± standard deviation (SD). For the statistical analysis, the SPSS version 12.0 software program (SPSS Inc., Chicago, IL, USA) was used, and the significance difference was verified at the 5% ($p < 0.05$) level with Duncan's multiple range test after one-way ANOVA.

3. Results

3.1. Evaluation of HAV Infectivity Using PMA/RT-qPCR after DBDP Treatment

For the PBS suspension results (Figure 1), the titer of the control of the non-PMA and PMA treatment groups was 3.87 copies/μL, and after 10, 20, 30, and 60 min of DBDP treatment, the non-PMA group were 3.67, 3.37, 3.10, and 2.72 copies/μL, respectively, showing a significant decrease as the treatment time increased ($p < 0.05$). In the PMA-treated group, the titers were 3.52, 3.19, 2.85, and 2.38 copies/μL, respectively, after 10, 20, 30, and 60 min of DBDP treatment. There was a significant difference between the non-PMA and PMA-treated sample at the treatment time of 30–60 min ($p < 0.05$). The D-value of the HAV of the PMA-treated suspension was 39.99 min, and R^2 was 0.97 (Figure 2).

The results of the reduction in titers in the PMA-treated and non-PMA-treated fresh oysters infected with HAV following treatment with DBDP are shown in Figure 3. In the case of the non-PMA group, the titer of the control was 2.68 log copies/μL, and after 10, 20, 30, and 60 min of DBDP treatment, the titers were significantly reduced to 2.48, 2.15, 1.89, and 1.57 log copies/μL, respectively ($p < 0.05$). In addition, even though DBDP was processed simultaneously, it was confirmed that there was a difference in the titers before and after PMA treatment. The titer of the control was the same, 2.68 copies/μL, and those after 10, 20, and 30 min of DBDP treatment, were 2.35, 2.05, and 1.75, respectively. The titers were further reduced in the non-PMA group at each time point. In particular, the titers decreased by 1.35 copies/μL after 60 min of DBDP treatment, and a significant difference ($p < 0.05$) between the non-PMA-treated and PMA-treated groups was shown. The D-value of the HAV in the PMA-treated oysters calculated by the first-order kinetics model was 46.73 min, and the R^2 for the model was 0.97, indicating high reliability (Figure 4).

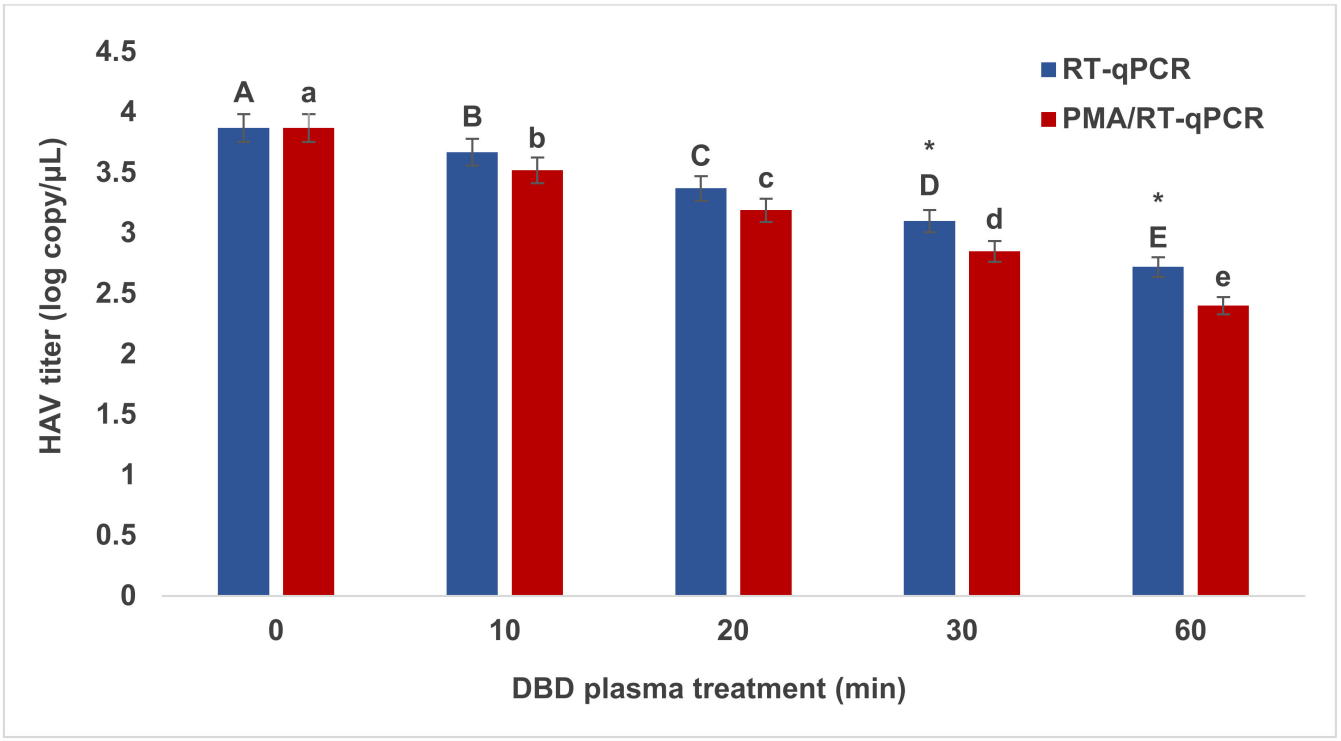


Figure 1. Comparison of HAV values in PBS suspension between RT-qPCR and PMA/RT-qPCR analysis by DBDP. Error bars indicate standard deviations of the mean of three samples per treatment. The different letters indicate significant differences at untreated samples (A–E) or PMA-treated samples (a–e) using Duncan's multiple range test ($p < 0.05$). Asterisks (*) indicate significant difference between untreated and PMA-treated samples by *t*-test ($p < 0.05$).

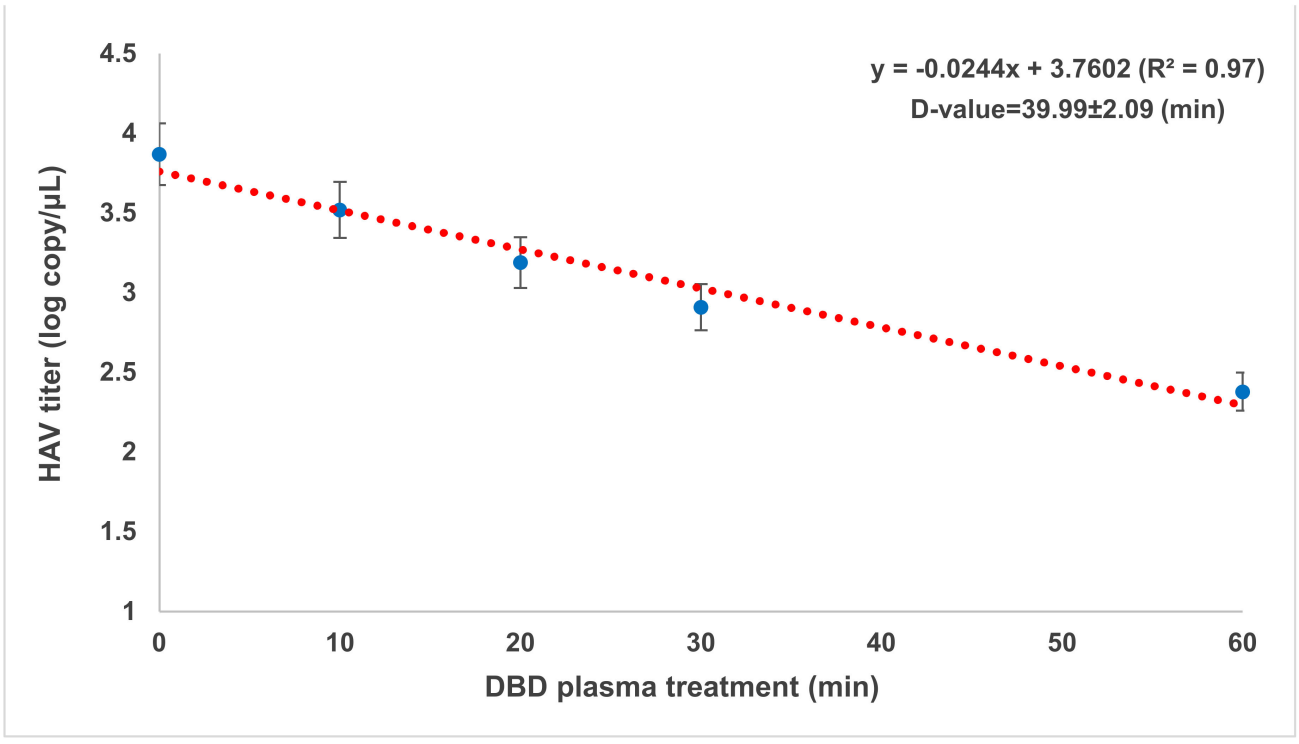


Figure 2. Fitted survival curves of HAV in PBS suspension treated by DBDP using the first-order kinetics model. Error bars indicate the standard deviations for the mean of three samples per treatment.

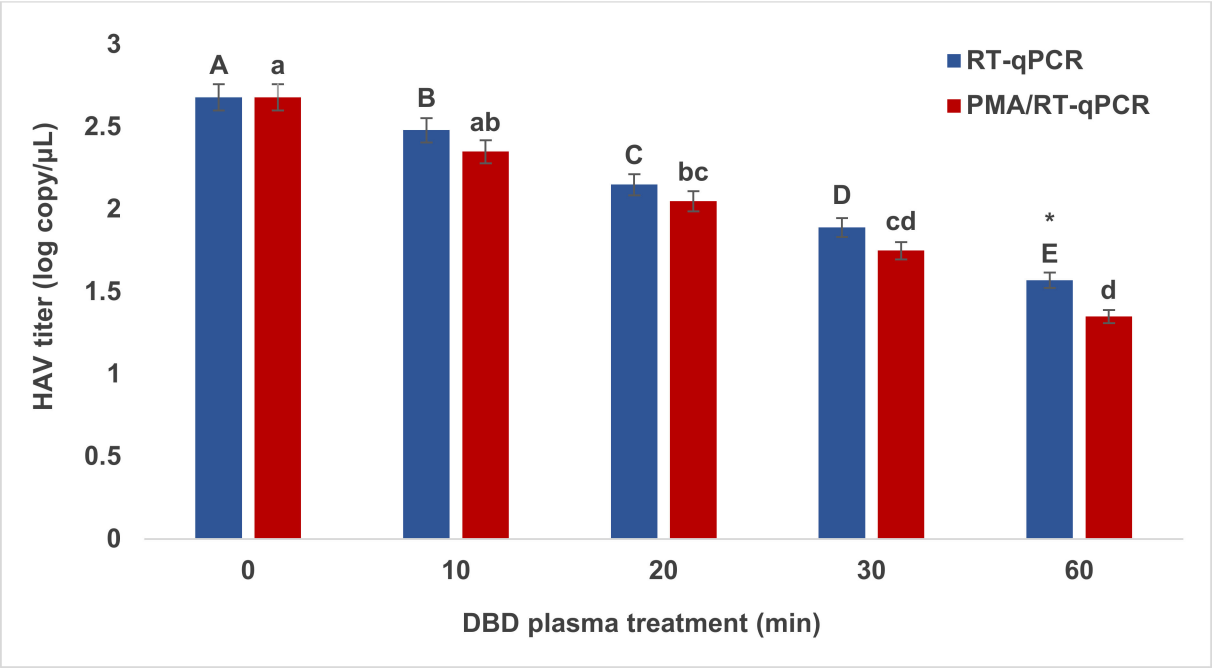


Figure 3. Comparison of HAV values in oyster between RT-qPCR and PMA/RT-qPCR analysis by DBDP. Error bars indicate standard deviations of the mean of three samples per treatment. The different letters indicate significant differences at untreated samples (A–E) or PMA-treated samples (a–d) using Duncan's multiple range test ($p < 0.05$). Asterisk (*) indicates a significant difference between untreated and PMA-treated samples by *t*-test ($p < 0.05$).

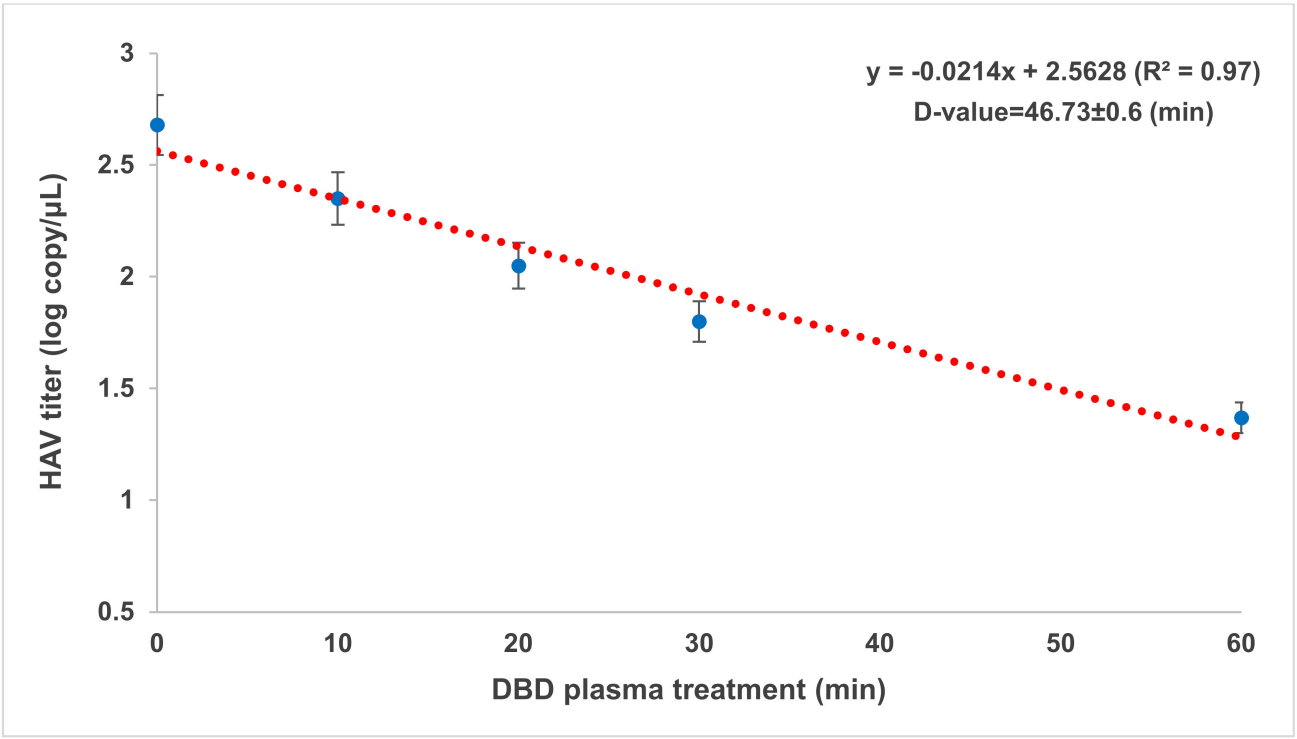


Figure 4. Fitted survival curves of HAV in oyster treated by DBDP using the first-order kinetics model. Error bars indicate the standard deviations for the mean of three samples per treatment.

3.2. Analysis of pH and Hardness of Fresh Oysters by DBDP Treatment

The change in the pH of fresh oysters following DBDP treatment is shown in Table 2. After 10, 20, 30, and 60 min of DBDP treatment, there was no significant difference between all groups ($p < 0.05$).

Table 2. pH value and hardness changes in the oyster treated by DBDP.

DBDP Treatment Time (min)	pH Value	Hardness (g/cm^2)
0	6.0 ± 0.01 ^{NS}	212.0 ± 3.0 ^{a}
10	5.9 ± 0.00	194.7 ± 3.8 ^{b}
20	5.9 ± 0.01	192.2 ± 8.7 ^{b}
30	6.0 ± 0.00	197.3 ± 2.8 ^{b}
60	6.0 ± 0.00	183.7 ± 6.9 ^{c}

The data indicates mean ± standard deviation (three samples per treatment). ^{NS} Non significant differences within the same column by Duncan's multiple range test ($p < 0.05$). The letters (a–c) of column indicate significant differences using Duncan's multiple range test ($p < 0.05$).

The hardness values obtained following the DBDP treatment are listed in Table 2. There was no significant difference at 10–30 min of treatment ($p < 0.05$), but there was a significant difference after 60 min of treatment ($p < 0.05$).

4. Discussion

Non-thermal sterilization technology minimizes the deterioration of the original quality of food because it sterilizes without applying heat; accordingly, the sterilization effect is also seen in the food industry. Non-thermal sterilization techniques include high-pressure processing [32], UV-C [33], ultrasonic [34], plasma treatment, and several other techniques. Plasma, a non-thermal sterilization technology, produced a chemically active species when it occurs, and it is similar to UV-C, a commonly known physical sterilization technology [35]. UV-C emits short wavelengths of 180–280 nm and has a high energy concentration, which has antibacterial effects [36]. In addition, treatment costs are low and chemical residues and heat are not generated [37], so it is mainly used as a sterilization technology for liquid foods [28]. However, UV-C has a small penetration depth, lamps are fragile, and the replacement cycle is short, which can adversely affect the human body owing to mercury and waste discharge. Moreover, the maximum effect cannot be observed at long warm-up times and low temperatures [38]. In comparison, DBDP is an eco-friendly technology that is widely used for medical purposes, such as cancer treatment [39] and dentistry [40], because it does not emit ingredients harmful to the human body. In addition, previous studies have demonstrated a sterilization effect on microorganisms. Bokhorst-van de Veen et al. [41] stated that cold atmospheric plasma can inactivate chemical and heat-resistant spores, and plasma is known to have a sterilizing effect on various microorganisms [29,42]. In addition, it is considered to have many advantages in sterilizing raw foods to minimize the deterioration of quality [43–45]. Therefore, in this study, sterilization was performed in suspensions and in oysters inoculated with HAV using DBDP, and the pH and hardness were measured to examine quality changes.

In suspensions and in oysters inoculated with HAV, the reduction in HAV by DBDP treatment was significantly different according to the DBDP treatment time. When comparing the control samples and the 60 min treated suspension and oyster samples, a maximum decrease of 1.15 and 1.11 log copies/μL was observed, respectively. There was a reduction of more than 1 log (90%) of HAV titers in both the suspension and oysters. Therefore, DBDP is thought to be effective in reducing HAV. Bae et al. [46] reported an up to 1.49 log copies/μL reduction in HAV titers in chicken breast using an atmospheric-pressure plasma jet. Shi et al. [47] also reported the effective deactivation of the hepatitis B virus using low-temperature plasma. Choi et al. [31] treated oysters with DBDP for 60 min to reduce the norovirus levels by up to 0.76 log copies/μL. Viral inactivation involves the breakdown of capsid proteins and nucleic acids. Changes in the shape of viral capsids alter

stability and induce damage to nucleic acids limiting viral replication in host cells [37]. The formation of active species in plasma destroys or changes the shape of the capsid protein, causing virus inactivation. It also damages capsid proteins, which enter in interior and damage nucleic acids, thereby preventing normal replication [48]. It has been reported that the inactivation of viruses using low-temperature plasma has successfully deactivated various viruses, such as the norovirus [49], adenovirus [50], and HAV.

RT-qPCR was used to detect HAV. RT-qPCR quantification is used as a detection method for diverse microorganisms, including viruses [51,52]. However, this method has the disadvantage of detecting both infectious and noninfectious viruses by amplification [53]. Nucleic acids from damaged viruses can persist for several months after the inactivation process [54,55], and RT-qPCR can eventually overestimate the number of viruses [56]. Therefore, it is necessary to distinguish between infectious and noninfectious viruses. In this study, a fluorescent dye, PMA, was used to distinguish between infectious and noninfectious viruses. PMA can only penetrate dead or membrane-damaged cells, and covalently binds to cell nucleic acids through photolysis. Consequently, nucleic acids become insoluble and the PCR amplification of nucleic acids from dead or membrane-damaged cells is inhibited [57]. In this study, the infection power was confirmed by the PMA treatment of the samples. In viral suspensions, there was a significant difference in titers between the PMA-treated and the non-PMA-treated samples in the 30–60 min treatment, and in the oysters infected with HAV, there was a significant difference at 60 min. PMA treatment allowed a distinction between infectious and non-infectious viruses, and an observation of the effect of DBDP treatment. Previous studies have reported that plasma treatment sufficiently damages the viral capsid, and thus, the PMA dye is well-attached to the inside to detect only infectious viruses, and it is effective against bacteria [58]. Choi et al. [31] also showed that the DBDP treatment of norovirus in oysters greatly helps to inactivate infectious viruses, and PMA preprocessing is an indirect method suitable for estimating the infectivity of viruses.

Since the 1980s, the consumer's interest in safe food has increased [59]. Therefore, the challenge in the food industry is to prevent or minimize quality degradation to produce high-quality products. pH and texture (hardness) are major factors that affect the quality of food. The pH affects the pigment, taste, texture, and shelf life of food, and rapid changes can have undesirable effects on food [60,61]. Texture (hardness) is an important factor that influences the perception of the sensory attractiveness of food. It represents freshness and contributes to the enjoyment of eating [62]. DBDP treatment did not affect the pH of oysters. Previous studies also reported that oysters and sea squirt did not have pH changes when treated with DBDP [47,49], and considering the results of this study, DBDP does not affect the pH of food. The hardness was significantly different from that in the control group. Sarangapani et al. [63] treated blueberries with atomic cold plasma and determined that plasma did not directly affect quality changes, such as texture. A number of studies using plasma reported no change in apples [64], melons [65], pork, beef [66], minced pork [67], and oysters [31]; the results of this study were different. This is could be due to the differences between individual oyster and the influence of the surrounding environment, and it is difficult to determine whether it is the effect of DBDP.

5. Conclusions

We demonstrated that DBDP can reduce HAV thresholds in suspensions and oysters. There was no difference in the pH of oysters after DBDP treatment, and the hardness seems to be due to the individual characteristics of oysters, not changed due to DBDP treatment. The results of this study focused only on the inhibitory effect of DBDP on the virus and the resulting quality change; therefore, continuous monitoring of oysters is required in future studies. It also suggests that reducing pathogenic microorganisms in food, while improving the treatment environment of DBDP and minimizing the deterioration of quality, may be a new solution for sterilizing food and ensuring safe consumption. The results of this study provide basic information to support the application of DBDP.

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References

1. Park, S.Y.; Ha, S.D. Thermal inactivation of hepatitis A virus in suspension and in dried mussels (*Mytilus edulis*). *Int. J. Food Sci. Technol.* **2015**, *50*, 717–722. [CrossRef]
2. Kim, H.S.; Oh, Y.H.; Thollot, Y.; Bravo, C. Post-marketing surveillance of hepatitis A virus vaccine (Avaxim® 160U) in south Korea from 2011 to 2015. *Infect Dis. Ther.* **2019**, *8*, 105–112. [CrossRef] [PubMed]
3. John, D.E.; Rose, J.B. Review of factors affecting microbial survival in groundwater. *Environ. Sci. Technol.* **2005**, *39*, 7345–7356. [CrossRef] [PubMed]
4. Nainan, O.V.; Xia, G.; Vaughan, G.; Margolis, H.S. Diagnosis of hepatitis a virus infection: A molecular approach. *Clin. Microbiol. Rev.* **2006**, *19*, 63–79. [CrossRef]
5. Hewitt, J.; Greening, G.E. Effect of heat treatment on hepatitis A virus and norovirus in NewZealand greenshell mussels (*Perna canaliculus*) by quantitative real-time reverse transcription pcr and cell culture. *J. Food Prot.* **2006**, *69*, 2217–2223. [CrossRef]
6. Sow, H.; Desbiens, M.; Morales-Rayas, R.; Ngazoa, S.E.; Jean, J. Heat inactivation of hepatitis A virus and a norovirus surrogate in soft-shell clams (*Mya arenaria*). *Foodborne Pathog. Dis.* **2011**, *8*, 387–393. [CrossRef]
7. Bidawid, S.; Farber, J.M.; Satter, S.A. Rapid concentration and detection of hepatitis A virus from lettuce and strawberries. *J. Virol. Methods* **2000**, *88*, 175–185. [CrossRef]
8. Long, S.M.; Adak, G.K.; O'Brien, S.J.; Gillespie, I.A. General outbreaks of infectious intestinal disease linked with salad vegetables and fruit, England and Wales, 1992–2000. *Commun. Dis. Public Health* **2002**, *5*, 101–105.
9. Le Guyader, F.S.; Schultz, A.C.; Haugarreau, L.; Crosi, L.; Maunula, L.; Duizer, E.; Lobber-Verschoor, F.; von Bonsdorff, C.H.; Suffredini, E.; van der Poel, W.M.; et al. Round-Robin Comparison of Methods for the Detection of Human Enteric Viruses in Lettuce. *Food Prot.* **2004**, *67*, 2315–2319. [CrossRef]
10. Seo, D.J.; Son, N.R.; Seo, S.W.; Lee, M.H.; Wang, X.Y.; Lee, J.S.; Joo, I.S.; Choi, C.S. Seafood and viruses. *Safe Food.* **2012**, *7*, 14–23.
11. Chintagari, S.; Hazard, N.; Edwards, G.; Jadeja, R.; Janes, M. Risks associated with fish and seafood. *Microbiol. Spectr.* **2017**, *5*, 123–142. [CrossRef] [PubMed]
12. Ki, M.R.; Son, H.J.; Choi, B.Y. Causes and countermeasures for repeated outbreaks of hepatitis A among adults in Korea. *Epidemiol. Health* **2019**, *41*, e2019038. [CrossRef] [PubMed]
13. Hansman, G.S.; Oka, T.; Li, T.C.; Nishio, O.; Noda, M.; Takeda, N. Detection of human enteric viruses in japanese clams. *J. Food Prot.* **2008**, *71*, 1689–1695. [CrossRef] [PubMed]
14. Wang, D.; Wu, Q.; Yao, L.; Wei, M.; Kou, X.; Zhang, J. New target tissue for food-borne virus detection in oysters. *Lett. Appl. Microbiol.* **2008**, *47*, 405–409. [CrossRef]
15. Sanchez, G.; Pinto, R.M.; Vanaclocha, H.; Bosch, A. Molecular characterization of hepatitis A virus isolates from transcomtinental shellfish-borne outbreak. *J. Clin. Microbiol.* **2002**, *40*, 4148–4155. [CrossRef]
16. Conaty, S.; Bird, P.; Bell, G.; Kraa, E.; Grohmann, G.; Mcanulty, J.M. Hepatitis A in new south wales, Australia, from consumption of oysters: The first reported outbreak. *Epidemiol. Infect.* **2000**, *124*, 121–130. [CrossRef]
17. Negara, B.F.S.P.; Mohibbullah, M.; Sohn, J.H.; Kim, J.S. Nutritional value and potential bioactivities of pacific oyster (*Crassostrea gigas*). *Int. J. Food Sci.* **2022**, *57*, 5732–5749. [CrossRef]
18. Suraiya, S.; Ahmmed, M.K.; Haq, M. Immunity boosting roles of biofunctional compounds available in aquafoods: A review. *Heliyon* **2022**, *8*, e09547. [CrossRef]
19. Jeong, S.H.; Kwon, J.Y.; Shin, S.B.; Choi, W.S.; Lee, J.H.; Kim, S.J.; Ha, K.S. Antibiotic resistance in shellfish and major inland pollution sources in the drainage basin of Kamak Bay, Republic of Korea. *Environ. Monit. Assess.* **2021**, *193*, 471. [CrossRef]
20. Fiorito, F.; Concilio, D.D.; Lambiase, S.; Amoroso, M.G.; Langellotti, A.L.; Martello, A.; Esposito, M.; Galiero, G.; Fusco, G. Oyster Crassostrea gigas, a good model for correlating viral and chemical contamination in the marine environment. *Mar. Pollut. Bull.* **2021**, *172*, 112825. [CrossRef]

21. Yu, Y.; Cai, H.; Hu, L.; Lei, R.; Pan, Y.; Yan, S.; Wang, Y. Molecular epidemiology of oyster-related human noroviruses and their global genetic diversity and temporal-geographical distribution from 1983 to 2014. *Appl. Environ. Microbiol.* **2015**, *81*, 7615–7624. [CrossRef] [PubMed]
22. Soni, A.; Smith, J.; Thompson, A.; Brightwell, G. Microwave-induced thermal sterilization- A review on history, technical progress, advantages and challenges as compared to the conventional methods. *Trends Food Sci. Technol.* **2020**, *97*, 433–442. [CrossRef]
23. Li, X.; Farid, M. Areview on recent development in non-conventional food sterilization technologies. *J. Food Eng.* **2016**, *182*, 33–45. [CrossRef]
24. Patel, J.; Parhi, A.; Al-Ghamdi, S.; Sonar, S.R.; Mattinson, D.S.; Tang, J.; Yang, T.; Sablani, S.S. Stability of vitamin C, color, and garlic aroma of garlic mashed potatoes in polymer packages processed with microwave-assisted thermal sterilization technology. *J. Food Sci.* **2020**, *85*, 2843–2851. [CrossRef] [PubMed]
25. He, J.; Wen, X.; Wu, L.; Chen, H.; Hu, J.; Hou, X. Dielectric barrier discharge plasma for nanometerials: Fabrication, modification and analytical applications. *TrAC Trends Anal. Chem.* **2022**, *156*, 116715. [CrossRef]
26. Domonkos, M.; Tich, P.; Trejbal, J.; Demo, P. Applications of cold atmospheric pressure plasma technology in medicine, Agriculture and food industry. *Appl. Sci.* **2021**, *11*, 4809. [CrossRef]
27. Herianto, S.; Hou, C.Y.; Lin, C.M.; Chen, H.L. Nonthermal plasma-activated water: A comprehensive review of this new tool for enhanced food safety and quality. *Copr. Rev. Food Sci. Food Saf.* **2021**, *20*, 583–626. [CrossRef] [PubMed]
28. Schneider, J.; Baumgartner, K.M.; Feichtinger, J.; Kruger, J.; Muranyi, P.; Schulz, A.; Walker, M.; Wunderlich, J.; Schumacher, U. Investigation of the practicability of low-pressure microwave plasmas in the sterilisation of food packaging materials at industrial level. *Surf. Coat. Technol.* **2005**, *200*, 962–966. [CrossRef]
29. Ryu, Y.H.; Uhm, H.S.; Park, G.S.; Choi, E.H. Sterilization of *Neurospora Crassa* by noncontacted low temperature atmospheric pressure surface discharged plasma with dielectric barrier structure. *J. Korean Vac. Soc.* **2013**, *22*, 55–65. [CrossRef]
30. Jung, M.I.; Park, S.Y.; Ha, S.D. Thermal inactivation of human norovirus on spinach using propidium or ethdium monoazide combined with real-time quantitative reverse transcription polymerase chain reaction. *Food Control* **2017**, *78*, 79–84. [CrossRef]
31. Choi, M.S.; Jeon, E.B.; Kim, J.Y.; Choi, E.H.; Lim, J.S.; Choi, J.S.; Ha, K.S.; Kwon, J.Y.; Jeong, S.H.; Park, S.Y. Virucidal effects of dielectric barrier discharge plasma on human norovirus infectivity in fresh oysters (*Crassostrea gigas*). *Foods* **2020**, *9*, 1731. [CrossRef] [PubMed]
32. Zhang, H.; Mittal, G.S. Effects of high-pressure processing (HPP) on bacterial spores: An overview. *Food Rev. Int.* **2008**, *24*, 330–351. [CrossRef]
33. Roy, P.K.; Mizan, M.F.R.; Hossain, M.I.; Han, N.; Nahar, S.; Ashrafudoulla, M.; Toushik, S.H.; Shim, W.B.; Kim, Y.M.; Ha, S.D. Elimination of *Vibrio parahaemolyticus* biofilms on crab and shrimp surfaces using ultraviolet C irradiation coupled with sodium hypochlorite and slightly acidic electrolyzed water. *Food Control* **2021**, *128*, 108179. [CrossRef]
34. Lin, L.; Wang, X.; Li, C.; Cui, H. Inactivation mechanism of *E. coli* O157:H7 under ultrasonic sterilization. *Ultrason. Sonochem.* **2019**, *59*, 104751. [CrossRef] [PubMed]
35. Brandenburg, R.; Lange, H.; Woedtke, T.V.; Stieber, M.; Kindel, E.; Weltmann, K.D. Antimicrobial effects of UV and VUV radiation of nonthermal plasma jets. *IEEE Trans. Plasma Sci.* **2009**, *37*, 877–883. [CrossRef]
36. Motakef-Kazemi, N.; Yaqubi, M. Green synthesis and characterization of bismuth oxide nanoparticle using *Mentha Pulegium* extract. *Iran. J. Pharm. Sci.* **2020**, *19*, 70–79. [CrossRef]
37. Hirneisen, K.A.; Black, E.P.; Cascarino, J.L.; Fino, V.R.; Hoover, D.G.; Kniel, K.E. Viral inactivation in foods: A review of traditional and novel food-processing technologies. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 3–20. [CrossRef]
38. Kim, S.J.; Kim, D.K.; Kang, D.H. Using UVC light-emitting diodes at wavelengths of 266 to 279 nanometer to inactivate foodborne pathogens and pasteurize sliced cheese. *Appl. Environ. Microbiol.* **2016**, *82*, 11–17. [CrossRef]
39. Keidar, M. Plasma for cancer treatment. *Plasma Sources Sci. Technol.* **2015**, *24*, 033001. [CrossRef]
40. Uhm, S.H.; Kwon, J.S.; Lee, E.J.; Lee, J.H.; Kim, K.N. Applications of non-thermal atmospheric pressure plasma in dentistry. *J. Korean Dent. Assoc.* **2014**, *52*, 783–794.
41. Bokhorst-van de veen, H.V.; Xie, H.; Esveld, E.; Abee, T.; Mastwijk, H.; Groot, M.N. inactivation of chemical and heat resistant spored of *Bacillus* and *Geobacillus* by nitrogen cold atmospheric plasma evokes distinct changes in morphology and integrity of spores. *Food Microbiol.* **2015**, *45*, 26–33. [CrossRef] [PubMed]
42. Sakudu, A.; Yagyu, Y.; Onodera, T. Disinfection and sterilization using plasma technology: Fundamentals and future perspectives for biological applications. *Int. J. Mol. Sci.* **2019**, *20*, 5216. [CrossRef] [PubMed]
43. Chen, Y.; Chen, G.; Wei, R.; Zhang, Y.; Li, S.; Chen, Y. Quality characteristics of fresh wet noodles treated with nonthermal plasma sterilization. *Food Chem.* **2019**, *297*, 127900. [CrossRef] [PubMed]
44. Rod, S.K.; Hansen, F.; Leipold, F.; Knochel, S. Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality. *Food Microbiol.* **2012**, *30*, 233–238. [CrossRef] [PubMed]
45. Shi, X.M.; Zhang, G.J.; Wu, X.L.; Ma, Y.; Shao, X.J. Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE Trans. Plasma Sci.* **2011**, *39*, 1591–1597. [CrossRef]
46. Bae, S.C.; Park, S.Y.; Choe, W.; Ha, S.D. Inactivation of murine norovirus-1 and hepatitis A virus on fresh meats by atmospheric pressure plasma jets. *Food Res. Int.* **2015**, *76*, 342–347. [CrossRef] [PubMed]
47. Shi, X.M.; Zhang, G.J.; Wu, X.L.; Peng, Z.Y.; Zhang, Z.H.; Shao, X.J.; Chang, Z.S. Effects of low-temperature plasma on deactivation of hepatitis B virus. *IEEE Trans. Plasma Sci.* **2012**, *40*, 2711–2716. [CrossRef]

48. Filipic, A.; Gutierrez-Aguirre, I.; Primc, G.; Mozetic, M.; Dobnik, D. Cold plasma, a new hope in the field of virus inactivation. *Trends Biotechnol.* **2020**, *38*, 1278–11291. [CrossRef]
49. Kim, J.Y.; Jeon, E.B.; Song, M.G.; Ha, K.S.; Jeong, S.H.; Jung, Y.J.; Park, S.Y. Combined of ultrasonic waves and dielectric barrier discharge plasma for the viable reduction in human norovirus while retaining the quality of raw sea squirt. *J. Food Process. Preserv.* **2021**, *44*, e13847. [CrossRef]
50. Sakudo, A.; Toyokawa, Y.; Imanishi, Y. Nitrogen gas plasma generated by a static induction thyristor as a pulsed power supply inactivated adenovirus. *PLoS ONE* **2016**, *11*, e0157922. [CrossRef]
51. Mackay, I.M.; Arden, K.E.; Nitsche, A. Real-time PCR in virology. *Nucleic Acids Res.* **2002**, *30*, 1292–1305. [CrossRef] [PubMed]
52. Hein, I.; Flekna, G.; Krassnig, M.; Wagner, M. Real-time PCR for the detection of *Salmonella spp*. in food: An alternative approach to a conventional PCR system suggested by the FOOD-PCR project. *J. Microbiol. Methods* **2006**, *66*, 538–547. [CrossRef]
53. Rudi, K.; Moen, B.; Dromtorp, S.M.; Holck, A.L. Use of Ethidium monoazide and PCR in combination for quantification of viable and dead cells in complex samples. *Appl. Environ. Microbiol.* **2005**, *71*, 1018–1024. [CrossRef] [PubMed]
54. Bemer, P.; Plouzeau, C.; Tande, D.; Leger, J.; Giraudeau, B.; Valentin, A.S.; Jolivet-Gougeon, A.; Vincent, P.; Corvec, S.; Gibaud, S.; et al. Evaluation of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: A prospective multicenter crosssectional study. *J. Clin. Microbiol.* **2014**, *52*, 3583–3589. [CrossRef] [PubMed]
55. Branger, S.; Casalta, J.P.; Habib, G.; Collard, F.; Raoult, D. *Streptococcus pneumoniae* endocarditis: Persistence of DNA on heart valve material 7 years after infectious episode. *J. Clin. Microbiol.* **2003**, *41*, 4435–4437. [CrossRef] [PubMed]
56. Askar, M.; Sajid, M.; Nassif, Y.; Ashraf, W.; Scammell, B.; Bayston, R. Propidium monoazide-polymerase chain reaction for detection of residual periprosthetic joint infection in two-stage revision. *Mol. Biol. Rep.* **2019**, *46*, 6463–6470. [CrossRef] [PubMed]
57. Nocker, A.; Cheung, C.Y.; Camper, A.K. Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. *J. Microbiol. Methods* **2006**, *67*, 310–332. [CrossRef]
58. Zhong, J.; Zhao, X. Detection of viable but non-culturable *Escherichia coli* O157:H7 by PCR in combination with propidium monoazide. *3 Biotech.* **2018**, *8*, 28. [CrossRef]
59. Van Boekel, M.A.J.S. Modelling of chemical reactions in foods: A multiresponse approach. *Acta. Hortic.* **1998**, *476*, 149–156. [CrossRef]
60. Andres-Bello, A.; Barreto-Palacios, V.; Garcia-Segovia, P.; Mir-Bel, J.; Martinez-Monzo, J. Effects of pH on color and texture of food products. *Food Eng. Rev.* **2013**, *5*, 158–170. [CrossRef]
61. Pankaj, S.; Wan, Z.; Keener, K.M. Effects of cold plasma on food quality: A review. *Foods* **2018**, *7*, 4. [CrossRef] [PubMed]
62. Wilkinson, C.; Dijksterhuis, G.B.; Minsekus, M. From food structure to texture. *Trends Food Sci. Tech.* **2000**, *11*, 442–450. [CrossRef]
63. Sarangapani, C.; O'Toole, G.; Cullen, P.J.; Bourke, P. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innov. Food Sci. Emerg. Technol.* **2017**, *44*, 235–241. [CrossRef]
64. Niemira, B.A.; Sites, J. Cold plasma inactivates *Salmonella Stanley* and *Escherichia coli* O157:H7 inoculated on golden delicious apples. *J. Food Prot.* **2008**, *71*, 1357–1365. [CrossRef] [PubMed]
65. Tappi, S.; Gozzi, G.; Vannini, L.; Berardinelli, A.; Romani, S.; Ragni, L.; Rocculi, P. Cold plasma treatment for fresh-cut melon stabilization. *Innov. Food Sci. Emerg. Technol.* **2016**, *33*, 225–233. [CrossRef]
66. Jayasena, D.D.; Kim, H.J.; Yong, H.I.; Park, S.; Kim, K.; Choe, W.; Jo, C. Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. *Food Microbiol.* **2015**, *46*, 51–57. [CrossRef]
67. Lee, J.; Jo, K.; Lim, Y.; Jeon, H.J.; Choe, J.H.; Jo, C.; Jung, S. The use of atmospheric pressure plasma as a curing process for canned ground ham. *Food Chem.* **2018**, *240*, 430–436. [CrossRef] [PubMed]

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