Decontamination of Sprouted Seeds by High Power Ultrasound and Irradiation

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Abstract

Novel technologies are investigated for replacing the chlorine to decontaminate fresh produce because of public health concerns and negative effect of chlorine to the environment. The objective of this study was to assess the decontamination efficacy of two state of the art technologies, high power ultrasound (US) and irradiation on mung bean and fenugreek sprouts. Firstly, mung bean seeds were treated with US (40 kHz, 240 W, 25 °C) for 5 min to evaluate US efficacy on seeds; while same parameters were applied for 5 and 10 mins for assessing the effect of time on decontamination impact on mung bean sprouts. Then, mung bean and fenugreek sprouts were exposed to ultrasound treatments (40 kHz, 240 W, 25 °C) for 10 min to investigate microbial reduction in total viable counts (TVC) and Enterobacteriaceae counts. Then, same products were treated with irradiation with doses of 1 and 2 kGy. There was no significant reduction in TVC and Enterobacteriaceae count on ultrasound treated fenugreek sprouts, while the same treatment resulted in significant reductions on mung bean sprouts. In contrast, irradiation treatment caused significant reductions in TVC and Enterobacteriaceae count in both products ($p < 0.05$). The microbial reductions on fenugreek sprouts were less than mung bean sprouts after the irradiation treatment, but they were still significant. The reductions with a dose of 2 kGy were higher than with a dose of 1 kGy. Irradiation treatment is promising for reducing the population of pathogens on sprouted seeds. On the other hand, ultrasound treatment proved to be not as effective as irradiation treatment.

Keywords: Decontamination, ultrasound, irradiation, sprouted seeds

Introduction:

Consuming fresh produce, including sprouts, is becoming much more popular in last decades because of healthy diet desire. In addition, vegetarian and vegan diets are also new trend in human nutrition. Therefore, the consumption of fruits and vegetables plays increasingly important role in human diet. However, there is also negative impact of eating fresh produce to the consumers. Since these products are eaten raw, contaminating pathogens can not be eliminated by effective decontamination methods like thermal processes. Sprouted seeds are one of the products at highest risk in the fresh produce industry. Sprouting conditions like high moisture and warm environment are also ideal for the growth of pathogens.

Foodborne outbreaks linked to fresh produce are being observed in recent years. For example, European Food Safety Authority (EFSA) reported an outbreak triggered by Shiga-toxin producing *Escherichia coli (STEC)* in Germany. Fenugreek sprouts were the cause of the biggest outbreak in Germany in the last 60 years which bring along 53 deaths (EFSA, 2011).

Decontamination of fresh produce is based on washing the products using water containing chemical substances and sanitizers (Gil et al., 2009). Nevertheless, there are concerns about usage of chemical substances because of their potential carcinogenic effect to humans, harm to the environment. That is why these compounds are banned in some European countries such as Sweden, Germany and Belgium (Ersus and Turantaş, 2013). The emerging technologies are investigated in order to replace these chemical sanitizers. High power ultrasound (US) and irradiation are the examples of novel technologies.

Ultrasound is a physical and non-thermal technique. Thus, it is deemed a safe decontamination method that can also maintain the organoleptic and nutritional characteristics of food, while it is considered as environmentally safe when compared to chemical substances and sanitizers. When ultrasound is performed over human hear limits, cavitation bubbles occur. These bubbles form a turbulence and high rates of shear during transient cavitation. Formation and collapsing of the bubbles near the surface creates microjets that have scour effect of the surface. This effect is the main effect for inactivation of microorganisms.

Food irradiation is one of the non-thermal treatments in order to decontaminate fruits and vegetables. Joint FAO/IAEA/WHO Study Group, (1999) considered that the irradiation of food up to an overall average dose of 10 kGy does not cause special nutritional and microbiological problems. Irradiation treatment for decontamination of microorganisms in foods occurs at medium doses (1-8 kGy).

In this study, we aimed to fill some of the knowledge-gaps on the efficacy of high power ultrasound and irradiation treatments for sprouts production. The aim of this study was to investigate the decontamination efficacy of the ultrasound and irradiation treatments on seeds and sprouts.

Materials and Methods

Commercial seeds intended for sprouting, decontamination treatments and microbiological analysis were purchased from market in Budapest, Hungary. For sprouting process, seeds were soaked into tap water for 5 hours. After that, they were replaced on a plastic box on a single layer with sterile spoon and tweezers. Filter paper was placed on the bottom of the boxes and water was added in order to keep the seeds in wet conditions. The seeds were watered after 24 hours. Then, the sprouts were large enough size after 48 hours in order to make treatment or microbiological analysis. The seeds were kept at room temperature in laboratory conditions.

Ultrasound treatment was implemented by ultrasonic cleaner bath (HBM Machines B.V., Netherlands) which has the capacity of 70 liters. Ultrasonic bath was filled with tap water. The samples were poured into the ultrasonic cleaner. The frequency and the power for all treatments on mung bean and fenugreek sprouts and seeds were 40 kHz and 240 W, respectively. Conditions were chosen based on previous studies on ultrasound treatment efficacy on seeds and sprouts (Ngnitcho et al., 2018; Scouten and Beuchat, 2002).

The bags containing sprouts were sealed and irradiated in 1 kGy and 2 kGy at a dose rate of 2 kGy/h. Gamma radiolysis experiments were carried out with PBq (2150 TBq) activity of ^{60}Co γ-source. Experiments were carried out in Hungarian Academy of Sciences Centre for Energy Research. The temperature during irradiation experiments was 20 °C. Samples were rotated during the experiments.

Just after the ultrasound treatments, the water was decanted and the samples were collected carefully with sterile spoon and tweezers and placed in sterile stomacher bags. For irradiation treatments, samples were collected after the treatments and the same procedure was implemented. 135 ml of 0.1% peptone water was added to each 15 g of sample and homogenized with a stomacher (BagMixer®, Interscience, France) for 2 min. Serial dilutions of homogenized seeds and sprouts samples were prepared with 0.1% peptone water. Proper dilutions were spread plated in triplicate onto Violet Red Bile Glucose (VRBG) Agar (Biokar, France) for the selective enumeration of Enterobacteriaceae, and Tryptone Glucose Extract (TGE) agar for the total viable count (TVC). TGE was prepared manually with the following formula: 0.5% bacteriological peptone (Biolab, Hungary), 0.25% yeast extract (Merck, Germany), 0.1% glycose (Reanal, Hungary) and 1.5% bacteriological agar (Biolab, Hungary). Samples for the enumeration of Enterobacteriaceae were incubated at 37°C for 24h, while the samples for TVC were incubated at 30°C for 24-48 h.

The results of analyses were statistically analyzed by ANOVA using the software SPSS 20 (SPSS, Inc., Chicago, IL) with the Duncan test to evaluate the differences between treatments at a level of significance $P < 0.05$.

Results and Discussion

According to previous research, pre-contamination of seeds is the most probable cause of sprout-associated outbreaks. National Advisory Committee on Microbiological Criteria for Foods, (1999) reported that sprout-associated outbreaks were mostly generated from the producers that does not perform seed disinfection treatments or use disinfectants at low concentrations. Thus, the committee recommends 5 log CFU/g microbial reduction on seeds intended for sprouting.

The total viable counts (TVC, CFU/g) on untreated seeds, seeds washed with water for 5 min and ultrasound (40 kHz, 240 W, 20 °C, 5 min) treated seeds are shown in Figure 1. Those viable counts correspond to the natural flora of the seeds. There was no significant reduction after washing the seeds for 5 min with water ($p > 0.05$). After the ultrasound treatment, there was a 0.85 log CFU/g reduction in the TVC compared to the control samples. Scouten and Beuchat, (2002) reported 0.73 and 0.55 log CFU/g reductions of inoculated *Salmonella* and E.*coli 0157:H7*, respectively, after the ultrasound (38.5-40.5 kHz, 5 min, 23 °C) treatment on alfalfa seeds.

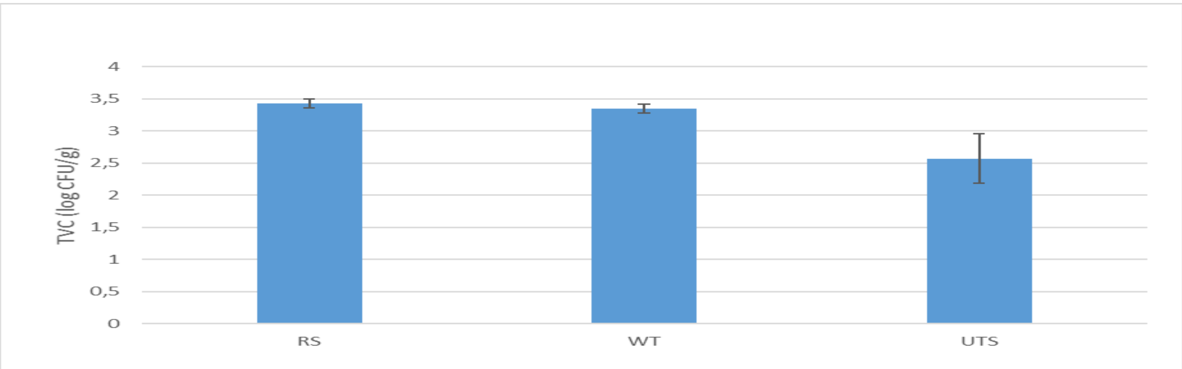

Figure 1. Total Viable Counts (log CFU/g) of control (RS), washed for 5 min (WT), ultrasound (40 kHz, 240 W, 25 °C, 5 min) treated (UTS) seeds. The error bars represent the standard deviation of the mean.

The current study and the previous study of Scouten and Beuchat, (2002) show that microbial reductions on seeds after ultrasound treatment are not even close to the recommended values (5 log CFU/g reduction). Therefore, ultrasound might be useful, but in combination with chemical sanitizers or other novel technologies. Specifically, the penetration capability of ultrasounds could enhance the efficacy of these treatments. For example, Scouten and Beuchat, (2002) obtained 2.27 and 1.94 log CFU/g reductions of *Salmonella* and E.*coli 0157:H7*, respectively, when they combined ultrasound (38.5-40.5 kHz, 240 W, 23 °C) treatment with 1% calcium hydroxide ($Ca(OH)_2$).

Total viable counts (TVC) and Enterobacteriaceae counts on mung bean sprouts before treatment (control), and after 5 and 10 min ultrasound (40 kHz, 240 W, 25 °C) treatments were investigated. After 2 days of sprouting treatment, both TVC and Enterobacteriaceae counts reached 8 log CFU/g on mung bean seeds. In the study of Splittstoesser et al., (1983), aerobic plate count (APC) and coliforms on violet red bile agar (VRBA) on mung bean sprouts reached 8.0 and 7.0 log CFU/g, respectively. Our results clearly not only confirm how sprouting provides optimal growth conditions for the rapid replication of potential pathogens, but also that the contaminants are likely to be already associated with the seeds to be sprouted as we germinated the seeds in sterile containers.

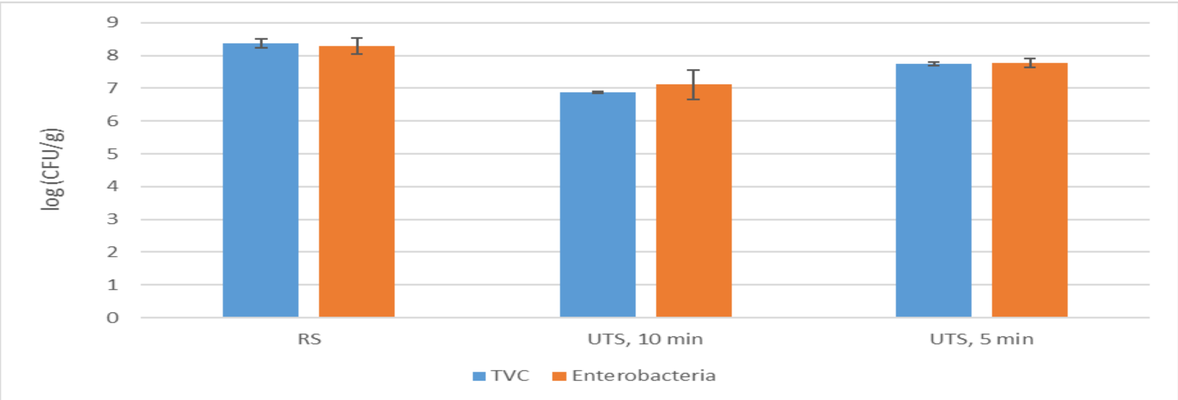

Figure 2. Total Viable Count (TVC) and Enterobacteriaceae counts of control (RS), ultrasound (40 kHz, 240 W, 25 °C, 10 min) treated (UTS, 10 min) and ultrasound (40 kHz, 240 W, 25 °C, 5 min) treated (UTS, 5 min) mung bean sprouts. The error bars represent the standard deviation of the mean.

The 10 min ultrasound treatment of mung bean sprouts resulted in 1.49 and 1.17 log CFU/g reductions of TVC and Enterobacteriaceae, respectively. When they were exposed to ultrasounds for 5 min, the reduction of TVC was 0.62 log CFU/g. Ultrasound treatment for 5 min did not cause significant reduction in Enterobacteriaceae count on mung bean sprouts. ($p > 0.05$). Scouten and Beuchat, (2002) investigated the effect of time during ultrasound (38.5 - 40.5 kHz, 200 W) treatment on alfalfa seeds. They concluded that there was no significant reduction of *Salmonella* and E. *coli* after 1st minute of treatment to further 9 minutes of treatment. In fact, from 2 min to 5 min, an increase in the *Salmonella* and E. *coli* populations was observed when the alfalfa seeds were treated with ultrasound at 23 °C. However, in our experiments, there was a decrease of both TVC and Enterobacteriaceae when the ultrasound treatment rose from 5 min to 10 min. The increase of the treatment length from 5 to 10 min caused significant reductions in TVC and Enterobacteriaceae on mung bean sprouts.

We subsequently assessed the reduction of TVC and Enterobacteriaceae on fenugreek sprouts after ultrasound (40 kHz, 240 W, 25 °C) treatment for 10 min. In light of the results obtained with the mung bean sprouts, fenugreek sprouts were only treated for 10 min since there was no significant reduction in Enterobacteriaceae after 5 min of ultrasound treatment on mung bean sprouts ($p > 0.05$). TVC and Enterobacteriaceae counts of untreated (RS) and ultrasound treated (UTS**) fenugreek sprouts are represented in Figure 3.

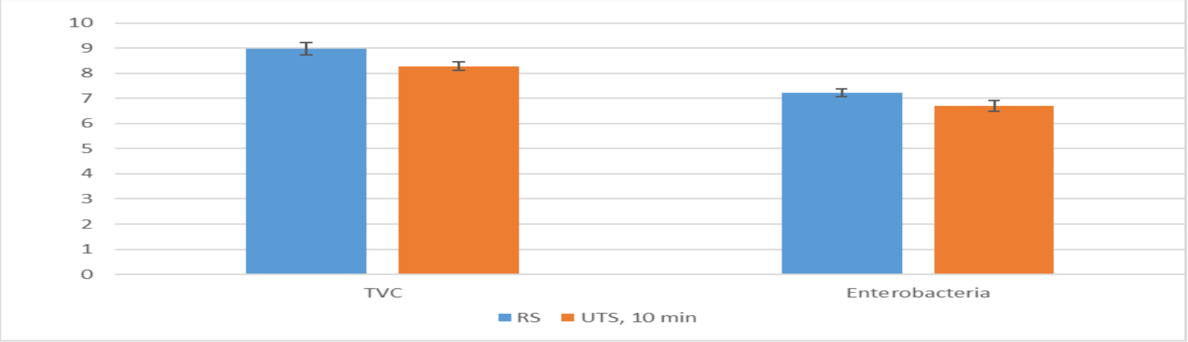

Figure 3. Total Viable Count (TVC) and Enterobacteriaceae counts of control (RS) and ultrasound (40 kHz, 240 W, 25 °C, 10 min) treated (UTS, 10 min) fenugreek sprouts. . The error bars represent the standard deviation of the mean.

Microbial reductions after 10 min of ultrasound (40 kHz, 240 W, 25 °C) on mung bean and fenugreek sprouts are illustrated in Figure 4. Our results show that the efficacy of the ultrasound treatment is higher on mung bean sprouts than on fenugreek sprouts. Ersus and Turantaş, (2013) reported that microbial reduction after ultrasound treatment on fresh produce varies between 0.5 and 1.98 log CFU/g. The efficacy depends on power, frequency, time and the temperature. In this study, the effect of time was studied on mung bean sprouts. Accordingly, increasing the treatment time from 5 min to 10 min caused significant reduction in TVC and Enterobacteriaceae counts on mung bean sprouts ($p < 0.05$). The power and the frequency were chosen respect to previous studies on mung bean, alfalfa and soybean sprouts (Millan-Sango et al., 2017; Ngnitcho et al., 2018). In additon, this study clearly shows that the efficacy of ultrasounds also depends on the type of product treated.

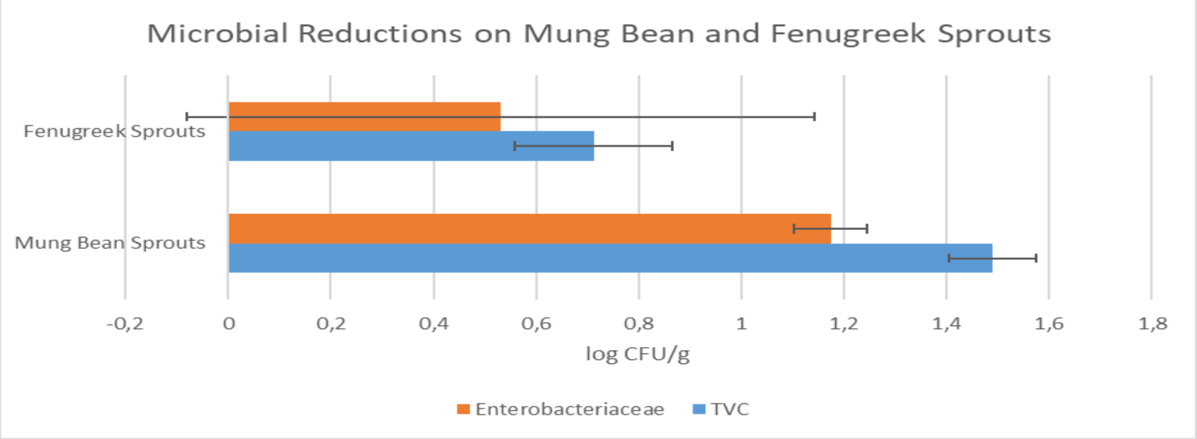

Figure 4. Microbial reductions (log CFU/g) on mung bean and fenugreek sprouts exposed to ultrasound (40 kHz, 240 W, 25 °C) treatment for 10 min. The error bars represent the standard deviation of the mean.

Overall, microbial reductions on both samples after ultrasound treatment were not dramatic. Ultrasound should therefore be applied in the combination with other technologies in order to have synergistic effects on the decontamination of the fresh produce.

The efficacy of 1 and 2 kGy irradiation treatments on mung bean sprouts was evaluated. The treatment resulted in 4.22 and 4.81 log CFU/g reductions of TVC, when mung bean sprouts were exposed to 1 kGy and 2 kGy irradiation treatment, respectively. For Enterobacteriaceae counts, the reductions were 4.29 and 5.65 log CFU/g for 1 kGy and 2 kGy, respectively. Irradiation treatment with the same doses of 1 and 2 kGy were also applied to fenugreek sprouts. The treatment resulted in 2.93 and 3.78 log CFU/g reductions of TVC, when fenugreek sprouts were exposed to 1 kGy and 2 kGy of irradiation, respectively. Irradiation treatment with a dose of 2 kGy caused 3.13 and 4.31 log CFU/g reductions in Enterobacteriaceae count on fenugreek sprouts.

Bari et al. (2004) also studied the effects of different doses of irradiation treatment on the eliminitation of *E. coli* O157:H7 and *Salmonella* on commercial ready to eat radish and mung bean sprouts. For mung bean sprouts, the irradiation treatment with a dose of 1 kGy caused 5.57 and 5.48 log CFU/g reductions in E. *coli* O157:H7 and *Salmonella*, respectively; while the reductions in 2 kGy dose were 5.97 and 5.48 log CFU/g for E. *coli* O157:H7 and *Salmonella*, respectively. One kGy treatments were enough to reduce *Salmonella* to nondetactable levels. For radish sprouts treated with a dose of 1 kGy, the reductions in E. *coli* O157:H7 and *Salmonella* were 3.30 and 3.14 log CFU/g, respectively; while a dose of 2 kGy resulted in 5.47 and 5.24 log CFU/g reductions of E. *coli* O157:H7 and *Salmonella*, respectively.

Microbial reductions of TVC and Enterobacteriaceae on mung bean and fenugreek sprouts are represented in Figure 5. Enterobacteriaceae are more sensitive to irradiation treatment. As we see on Figure 5, the reductions on mung bean sprouts are greater than on fenugreek sprouts with the doses of 1 kGy and 2 kGy. Indeed, microbial reductions on both sprouts are promising.

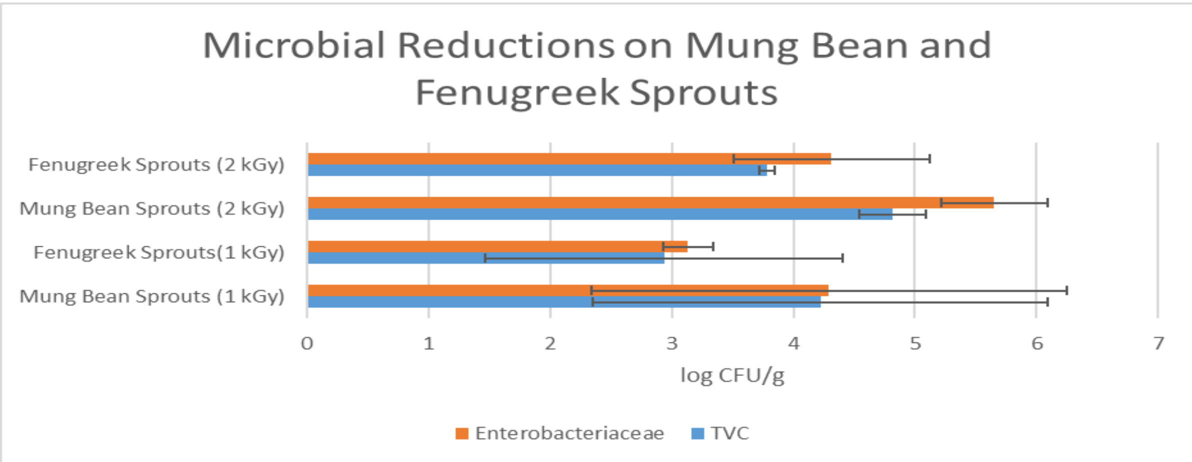

Figure 5. Microbial reductions (log CFU/g) on mung bean and fenugreek sprouts exposed to irradiation treatment with doses of 1 kGy and 2 kGy. The error bars represent the standard deviation of the mean.

Researchers must take into consideration that regulations impose limits on the radiation dose that can be used on fresh produce. In the U.S, the maximum dose for fresh produce including sprouts is 1 kGy (FDA, 2017)**.** Therefore, even if there was significant difference in the reduction of total viable count and enterobacteriaceae between the doses of 1 and 2 kGy, irradiation treatment with a dose of 2 kGy is above the limits. But, microbial reduction of irradiation treatment with a dose of 1 kGy is still promising. The combination of irradiation treatment with other technologies should be studied for further research.

Conclusions

Ultrasound treatment did not cause significant reduction in TVC and Enterobacteriaceae counts on fenugreek sprouts, when fenugreek sprouts were treated for 10 min. In contrast, the same treatment resulted in significant reductions of TVC and Enterobacteriaceae on mung bean sprouts ($p < 0.05$). Therefore, it can concluded that the efficacy of treatments depends on the product and the microbial group. This study has shown that irradiation treatment is an efficient method for the decontamination of mung bean and fenugreek sprouts. Its effect on the reduction of Enterobacteriaceae is greater than of total viable count (TVC) in both mung bean and fenugreek sprouts. This suggests that Enterobacteriaceae are more susceptible to this decontamination method than other microbial groups. Microbial enumerations showed that irradiation treatment was more effective in the reductions of TVC and Enterobacteriaceae counts on mung bean sprouts than on fenugreek sprouts. Overall, irradiation treatment was effective in microbial reductions on mung bean and fenugreek sprouts. Further research should be carried out on combining irradiation treatment at a maximum dose of 1 kGy with other novel technologies. Microbial reductions resulting from the ultrasound treatment were smaller compared to irradiation, indicating it is not an effective technology to be used alone in the decontamination of sprouted seeds. Further research should be performed on the enhancing effect of ultrasound technology on chemical sanitizers or other novel technologies.

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