Disinfection of *E. coli* and Removal of Pesticide Residues on Fresh Chili by Micro-bubble Plasma Ozonation

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ABSTRACT

The objectives of this experiment were to study effect of high voltage power supply, air flow rate on plasma dielectric barrier discharge (DBD) system and developed micro-bubble plasma ozonation approach to produce amount of ozone concentration and to use ozone for disinfection of microorganism (*E. coli*) and pesticide residues on fresh green chili. The ozone concentration generated by the plasma DBD was evaluated by the standard KI method. The AC high voltage (HV) power supply varied between 6 and 10 kV with air flow rate ranging of 6-10 l/min. The results showed that ozone concentration relatively depended on HV supply and air flow rate. The suitable HV across electrodes for generating ozone was 10 kV at the air flow rate of 10 l/min. Operating time for treatment of the chili was varied between 10 and 40 min. The chili was immersed in piped water (PW), Potassium permanganate solution (PPS), Bisodium carbonate solution (BCS) and ozonated water and all of results were comparative studied in terms of *E. coli* disinfection and removal of pesticide residues on chili. The amount of *E. coli* was determined by Multi Tube Fermentation (MTF) technique whilst the pesticide residues were analyzed by MJPK test kit and Gas Chromatography (GC). The experimental results showed that for laboratory scale experiment using 1 kg of chili treatment by ozonated water comparing to other treatments (PW, PPS and BCS), the dissolved ozone generated by 10 kV could reduce *E.coli* from 240 MPN/g to 10 MPN/g and the pesticide residues on chili were also in the safe level scale for 40 min of duration time. In addition, the demonstration scale experiment for 20 kg of chili treatment by ozonated water deactivate *E.coli* from 110,000 MPN/g to 46,000 MPN/g and reduced pesticide residues from 0.023 mg/kg to <0.010 mg/kg in 40 min of duration time. Ozonation system had relatively high potent both of deactivate *E. coli* and reduce pesticide residues in fresh chili compared to the other treatment and had a low electrical energy consumption (0.8 kWh on discharge time of 40 min).

KEYWORDS: *E. coli*, Ozone, Pesticide, Plasma dielectric barrier discharge

Introduction

Due to population growth every year, food and agricultural products must be enough to supply and are also safe for people. It is realized that the world population will be ten billion people in 2050, which is the main reason for producing the food and agricultural product security for them. The big problem is the food quality control even science and technology process can increase agricultural productivities and control food qualities (Chawla, Kaushik, Shiva Swaraj, & Kumar, 2018). At present, agriculture farming will rely on the use of pesticides and other chemicals to increase agricultural productivity and preventing of pests disturbance. The World Health Organization (WHO) seriously reported the impurities and pesticide residues on agricultural products in many countries around the world (Eliasson, Hirth, & Kogelschatz, 2000; Möhring, Gaba, & Finger, 2019). Additionally, decontamination of microorganism and the pesticide residues in food and agricultural products also are harmful to human such as *Escherichia coli* (*E.coli*), Organophosphate and Organochlorine compounds etc. The *E. coli* is one of the pathogenic bacteria in water and it can threaten people's health. Fortunately, *E. coli* bacteria can be controlled and destroyed by many methods for example chlorination, UV radiation, heating etc. However, the chlorination induce the secondary hazardous pollutants on water such as trihalomethanes and haloacetic acids whilst use of UV radiation depended on surrounding temperature and relative humidity (Yao *et al*., 2018). To compare to other sterilization techniques, the non-equilibrium plasma discharge (temperature of each particle in plasma is different, normally electron temperature is relatively high compared to the ion charges and neutral atoms) is a superior alternative allowing to reduce these risks and drawbacks, especially on producing of ozone and UV radiation (Chawla *et al*., 2018; Eliasson *et al*., 2000; Yao *et al*., 2018)

Ozone is an oxygen allotrope of which three atoms bound together to form one ozone molecule. Ozone is a good example for an industrial process of plasma dielectric barrier discharge (DBD) application to utilize tools of modern plasma physics (Eliasson *et al*., 2000). The conventional plasma DBD method use for producing ozone is supplied by various power supply such as AC high voltage power supply, high frequency power supply and electromagnetic power source etc. (Eliasson *et al*., 2000; Boonduang *et al*., 2012; Jodzis & Zieba, 2018). Due to the ozone oxidizing power, numerous applications on science and technology researches have been represented on disinfection of some products, potent germicidal properties, high oxidation potential of water and wastewater treatment etc. (Eliasson *et al*., 2000; Möhring *et al*., 2019). Consequently, it easily self decomposes and oxidizes matter without toxic by-product to the environment. However, ozone needs to be produced on site because of its own decomposition, especially on warm weather surrounding conditions (exothermic reaction). Ozone has been proven to be a substance capable of storing agricultural products because of its capability to destroy toxins from microorganisms and pesticides. In general, agricultural products use piped water (PW), Potassium permanganate solution (PPS) and Bisodium carbonate solution (BCS) to remove residue (Eliasson *et al*., 2000; Tang, Zhuo, Cui, Li, & Yao, 2019; Yao *et al*., 2018). At the present, ozone treatment is a few research applications to vegetable, fruit and food products even the pesticide residues and infection of microorganisms in agricultural products is an important issue that affects both humans and the environment, especially in Thailand.

Therefore, the aim of this research were to study physical parameters affecting to ozone synthesis on plasma dielectric barrier discharge tube and to develop compact ozone micro-bubbling system for inactivation of *E. coli* bacteria and reduce the toxic pesticide residues on fresh chili on laboratory scale and demonstration scale for small and medium enterprises.

Research Objectives

The main objectives of this work were to study the effect of high voltage power supply and air flow rate on plasma dielectric barrier discharge (DBD) system to produce amount of ozone concentration and to use ozone for disinfection of microorganism (*E. coli*) and pesticide residues on chili.

Benefit of Research

Obtain an ozone generator with the right concentration. For use to reduce the amount of *E. coli* and pesticide residues in chili.

Research Process

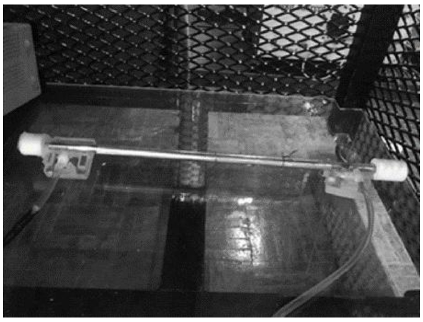
1. Plasma ozonizer system. This power supply unit consists of a variable transformer (so-called variac), a dump resistor for limiting electric current flow and AC voltmeter. Figure 1(a) and 1(b) shows the ozone cell and power supply unit, respectively. The power supply unit which produces a 50 Hz high voltage output ranging of 0-15 kV is designed and built in the case as shown in figure 1(b). Figure 2 illustrates the electrical circuit diagram of the power supply unit and its measurement. When the high voltage supplies both electrodes, the electrical energy across electrodes will ionize oxygen in air between electrode gap and produce the ozone molecules and some radical species of gases. The reaction for ozone synthesis is an endothermic reaction and the overall exothermic reaction of ozone generation is written as follows equation (1) (Eliasson *et al*., 2000)

$$3O_2 \longrightarrow 2O_3 \qquad (1)$$

(At 1 atm, + 284.5 kJ/mol)

The ΔH° at atmospheric pressure is +284.5 kJ/mole and its redox potential is +2.07 V which is in a group of acute toxicological effect. Since the standard Gibb's free energy of ozone formation ΔG° at 1 atm is +161.3 kJ/mole so ozone can be rapidly decomposed by heating. That is the reason why ozone synthesis needs to be run on-site (Eliasson *et al*., 2000).

a)



b)

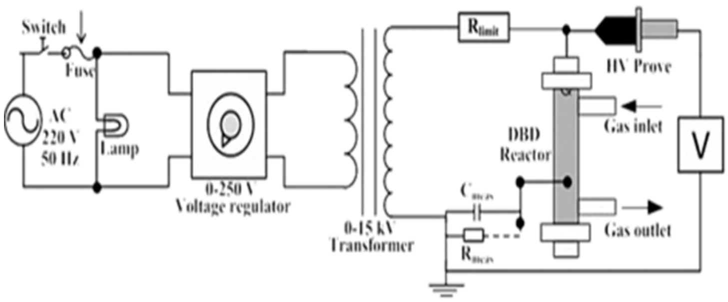


Figure 1. Illustration of set-up of the DBD plasma ozonizer system. a) Dielectric barrier discharge tube for generating of ozone (PSU-O3#2) ; b) 0-15 kV AC power supply for dielectric barrier discharge tube.

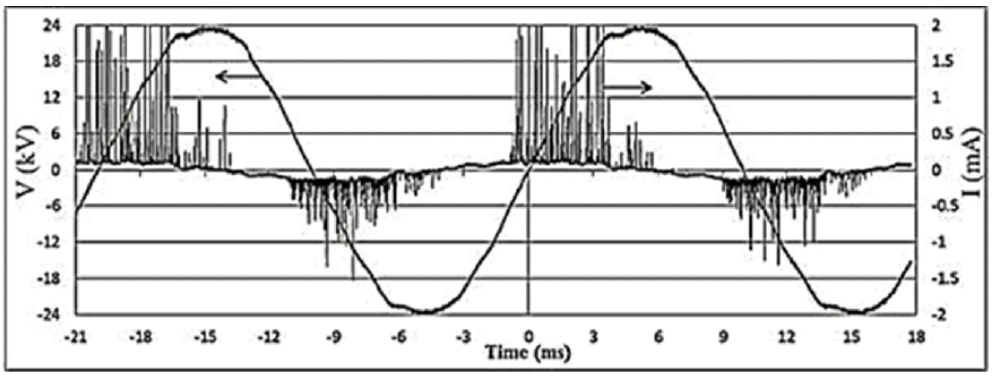


Figure 2. Voltage and current-waveforms of O_2 plasma dielectric barrier discharge.

2. Determination of ozone concentration. The amount of ozone concentration is determined by standard KI method. By preparation of 1% KI absorbing reagent solution in 0.1 M Phosphate buffer by weighing KH_2PO_4 of 13.61 g, KI of 10.00 g and anhydrous disodium hydrogen phosphate 14.20 g. Dissolve all chemicals substances in the deionized water, resulting in a volume of 1 liter was placed at room temperature for 1 day before use.

Preparation of standard iodine solution 0.025 M was carried out by weighing KI of 16.0 g and I of 3.1730 g. Dissolve all chemical substances in the double distilled water, making a volume of 500 ml. Storing this stock solution at room temperature for at least 1 day before use

To make standard calibration curve of amount of iodine solution versus UV absorbance, preparing of 0.00125 M iodine solution by mixing with the absorbing reagent solution 5 ml volume of 0.025 M Iodine and a volume of 100 ml volume 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml dissolved with absorbing reagent solution until the volume is 25 ml. Take each various standard iodine solution to measure the absorbance by UV-VIS Spectrophotometer (Brand HITACHI Model U – 2900) at 352 nm a curve between absorbance and iodine concentration was plotted. Since one mole of ozone react to two moles of potassium iodide solution and get one mole of iodine as following equation (2). Therefore, the standard calibration curve can be used for determination of the ozone concentration directly.

$$O_3+2KI+H_2O \longrightarrow I_2+2KOH+O_2 \qquad (2)$$

3. Effect of the plasma ozonizer parameters on ozone synthesis. Due to previous research works, there are many physical parameters which affect to ozone produced such as power supply, air or gas flow rate, electrode spacing gap, electrode length and gas species etc. (Eliasson, Hirth, & Kogelschatz, 2000; Boonduang *et al.*, 2012). Therefore, the effect of air flow rate, high voltage supply and discharge time on ozone concentration are studied because the value is not fixed, can be changed and presented in this works while the electrode spacing gap is fixed at the 3 mm gap width which is referred on the previous work (PuengKum, & Tirawanichakul,2011. Firstly, the compressed air flow rate was regulated by the air pump. The air flow rate varies from 6 l/min to 10 l/min. Secondly, the AC high voltage supply to ozonizer tube is varied between 6 and 10 kV and the amount of ozone concentration is evaluated by dissolving ozone with the microscale ceramic diffuser into absorbing reagent in each operating conditions for 0.5, 1.0, 1.5 and 2.5 min. And the voltage and current during run experiment are measured by HV probe and current probe, respectively.

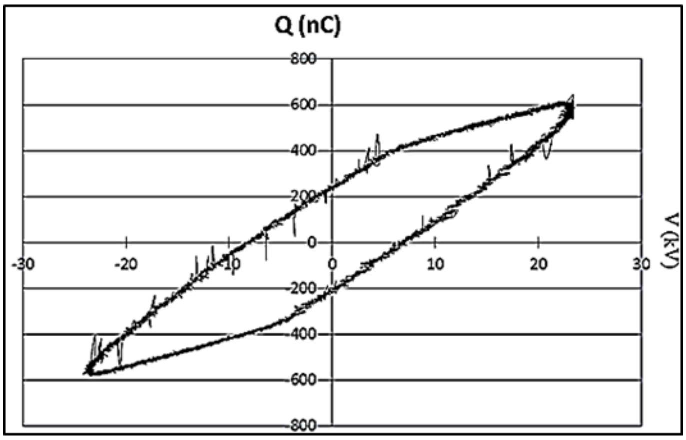


Figure 3. The Q-V oscillographic presentation (Lissajous figure)

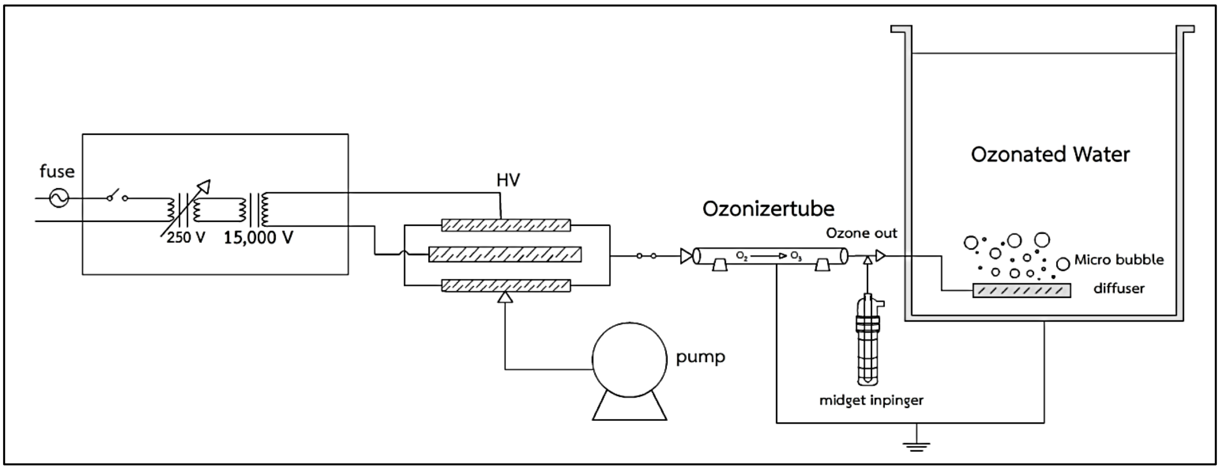


Figure 4. Schematic diagram of experimental set-up (The size of the tank is 0.5 m wide, 1 m long and 0.4 m high and uses 100 liters of water).

4. Disinfection of *E. coli* bacteria. To study disinfection and deactivation of *E. coli* bacteria on fresh chili before and after treatment because *E. coli* is often found in vegetables, especially chili, the experiments are carried out by submerging the chili of 1 kg and 20 kg in the ozone dissolved tank by varying discharge times of 0, 10, 20, 30 and 40 min. Suitable high voltage value and air flow rate from the previous subsection 3 were applied and used for this section. The ozonation treatment on chili was compared with soaking with piped water, Potassium permanganate solution and Bisodium carbonate solution (The common methods used for washing vegetables). The chili samples will be randomly taken and analyzed by the Science central laboratory unit of the Faculty of Science, Prince of Songkla University, Thailand for evaluation of *E. coli* reduction in each duration period.

5. Removal of pesticide residues. To study removal of pesticide residues on fresh chili after ozonation treatment, the experiment is carried on by submerging the fresh chili sample of 1 kg and 20 kg of weight in the ozone dissolved tank by varying duration times of 0, 10, 20, 30 and 40 min. Giving the voltage of the alternator to 10 kV and air flow rate to 10 l/min compared to submerging the fresh chili sample with piped water, Potassium permanganate solution and Bisodium carbonate solution. The chili will be tested after each treatment by pesticide residues test kit for 1 kg of chili (laboratory scale) and by GC analysis for 20 kg of chili (demonstration scale). The chili sample will be random taken and sent to Central Laboratory (Thailand) Co., Ltd. for determination of the amount of pesticide residues.

6. Specific energy consumption analysis. During ozone treatment in each experiment condition, the electric energy consumption was measured by watt hour meter (Brand K.Y. GROUP ELECTRICS) and was used for calculation energy consumption.

Instruments

The plasma ozonizer system (PSU-O3#2) consists of 0-15 kV AC high voltage power supply unit, air pump and ozone tube as shown in figures 1(a) and 1(b). The ozone was generated by passing compress air across high electric field of electrodes gap in the tube and the ozone concentration is precisely determined by following the standard KI method (ASTM, 1995). The voltage across DBD electrode (V) and current (I) are measured by a high voltage divider probe of Tektronix model P6015A and pulse current probe CT-1 (Tekronix Co. Ltd. USA). At the same time, a voltage probe (IWATSU, model: SS-0110) is used for measuring the discharge voltage (V_r) across on R_{meas} and C_{meas} (V_c). These V-I signals are recorded by the Digitized Storage Oscilloscope (Tektronix, model: TDS3014B).

The amount of *E. coli* is determined by Multi Tube Fermentation Technique (MTFT). The pesticide residues are analyzed by MJPK test kit and Gas Chromatography method. Reference test method Including Oraganophosphate Group use In-house method TE-CH-031 based on Steinwandter, Organochlorine Group use In-house method TE-CH-030 based on Steinwandter, Carbamate Group use In-house method TE-CH-032 based on Steinwandter and Pyrethroid Group use In-house method TE-CH-030 based on Steinwandter. The more detail will be discussed in the Results and Discussion.

Results and Discussion

1. Determination of ozone concentration. By following the KI methods in determination of ozone concentration, the ozone concentration can be relatively directly determined with the KI solution changing to iodine. Figure 5 illustrates the calibration curve of ozone concentration by following the standard KI methods of potassium iodide. The results show that the absorbance of light is linearly proportional to ozone concentration and can be written as the following equation (3)

$$Ab = 12.398x + 0.0686 \qquad (3)$$

$$r^2 = 0.9939$$

Where Ab = Absorbance (a.u.)

x = Ozone concentration (μmole/l)

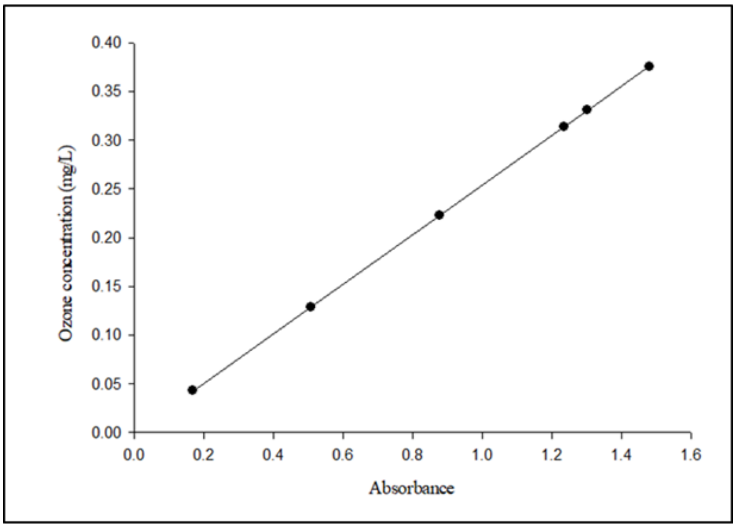


Figure 5. Calibration curve between the ozone concentration (directly proportional to standard potassium iodine (KI) concentration) and light absorbance.

2. Effect of the plasma ozonizer parameters on ozone synthesis. The current and voltage signals are measured by the HP high voltage divider probe and the current probe as shown in figure 2. The ozonizer plasma DBD tube is operated using the applied 50 Hz voltage at 11, 15, 19 and 23 kV with air flow rate of 10 l/min. The results show that the voltage frequency is the same as normal AC voltage of 50 Hz while the discharge current pattern appears in the plasma DBD tube of O_2. The many peaks of the discharge current are generated by the rate of gas dissociation. Moreover, the amount of each current peak lets oxygen and other gas species molecules to ionize and form ozone molecules and some nitrogen oxide molecules. The peak density has an obvious density at 23 kV. The high density of discharge peak is mentioned in the high gas dissociation and chemical reactions (Kogelschatz, 2003). Therefore, the numerous discharge peaks are the important role for referring to the increasing rate of gas dissociations and chemical reactions. (Eliasson *et al*., 2000; Kogelschatz, 2003). To determine in which mode of the DBD system is operating for each discharge gas, voltage and current waveform are acquired. The discharge current can be obtained by dividing V_r by

R_{meas}, while the charge transfer (Q) can be obtained by multiplying V_c by C_{meas}. Plotting Q-V diagram is illustrated as shown in figure 3 (so-called Lissajous figure). This figure is used to determine the discharge power (P) in the DBD reactor. The discharge power is then calculated by multiplying the Q-V area with the frequency of the applied voltage. Because the electric energy consumed per voltage cycle (E) is equal to the area of Q-V diagram (Wagner *et al.,* 2003). The total charge transfer which has influenced the discharge power depends on the gas properties, the discharge gap and the properties of the dielectric matter. The discharge power can be determined by Lissajous figure for each applied voltage while the energy density can be evaluated by dividing the discharge power by the flow rate of gas (Yoshida, Rajanikanth & Okubo, 2009). Thus, the discharge power and energy density of this DBD tube is in ranges of 0.1-0.7 eV and 1-4.4 kJ/l, respectively. The highest discharge power and energy density of O_2 are 0.73 W and 4.4 kJ/L, respectively.Due to the study effect of air flow rate and high voltage supply on ozone synthesis, the experimental results from figure 6 and figure 7 show that the ozone concentration produced from this plasma DBD tube is directly proportional to air flow rate and power supply and discharge time. The duration time reaction for ozone synthesis is in millisecond thus oxygen molecules can be dissociated and formed ozone molecules in a short period. So the high air flow rate can increase the oxygen content passing through the discharge gap between electrodes which are supplied by high voltage supply. Additionally, due to increase of the electric potential from voltage supply and discharge time, a high electric field between electrodes provides more electrons to break down the oxygen molecules. And the increase of discharge time has also increased feasibility synthesis ozone molecules among high voltage supply. These results are corresponded to the previous research works. The amount of air flow and voltages affect the amount of ozone. (Eliasson *et al.*, 2000; Boonduang, Limsuwan, Kongsri, & Limsuwan, 2012; Wei, Peng, Li, & Zhang, 2016; Jodzis & Zieba, 2018).

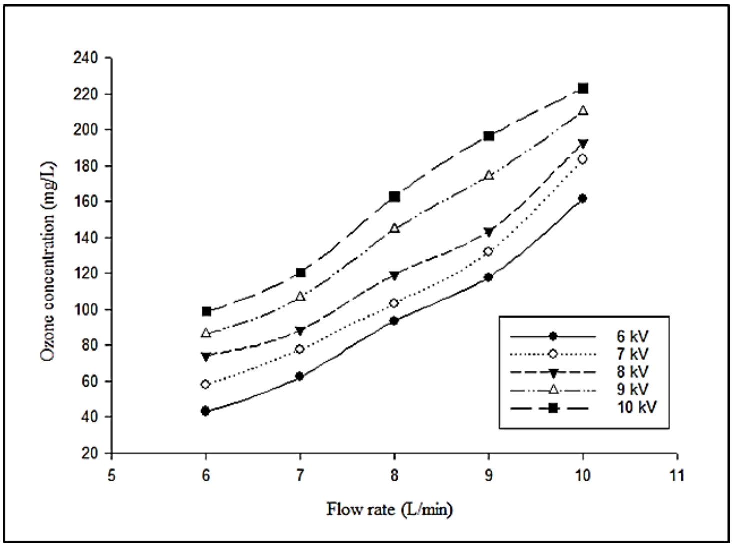


Figure 6. Ozone concentration produced versus air flow rateat high voltage supply ranging of 6-10 kV

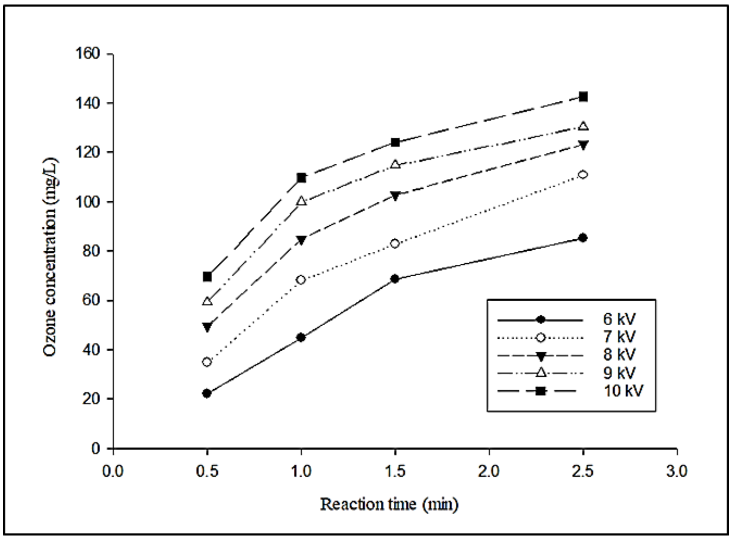


Figure 7. Ozone concentration among reaction time at high voltage ranging of 6-10 kV.

3. Deactivation of *E. coli* bacteria. From the study disinfection of *E. coli* by ozonation time at 10 kV and air flow rate 10 l/min, the results are shown in figure 8, Table 1 and Table 2. The results state that the reduction of *E .coli* bacteria has been significantly affected by ozonation with micro-bubbling. The increase of high voltage is efficient for deactivation *E. coli* because it provides a stronger electric field to generate active oxygen species or nitrogen species. In addition, the oxygen species, ozone and nitrogen species act on the bacteria cause disruption of the membrane stability and may inactivate enzymes (Zhou, 2016). Thus the applied high voltage is one of the most powerful parameters on the bacteria deactivation.

The experimental results of this work correspond to the previous research works. Ozone can reduce the amount of *E. coli*. (Arjunan *et al*., 2009; Gomes *et al*., 2018; Rodriguez-Mendez *et al*., 2013). Finally, to comparative study with other deactivation methods mentioned above, submerging 1 kg and 20 kg of chili sample with ozonated water can reduce the amount of *E. coli* more than those of submerging with piped water, Potassium permanganate solution and Bisodium carbonate solution. The results are presented on Table 1 and Table 2, respectively.

Table 1. Total *E. coli* amount on each treatment on 1 kg of fresh chili sample

Samples	*E. coli* (MPN/g)
Raw chili (unwashed sample)	240
Chili washed with piped water	230
Chili washed with Potassium permanganate water dissolved	180
Chili washed with Sodium bicarbonate water dissolved	160
Chili washed with ozonated water discharge for 10 minutes	150
Chili washed with ozonated water discharge for 20 minutes	110
Chili washed with ozonated water discharge for 30 minutes	60
Chili washed with ozonated water discharge for 40 minutes	10

Table 2. Total *E. coli* amount on each treatment on 20 kg of fresh chili sample

Samples	*E. coli* (MPN/g)
Chili washed with piped water	110,000
Chili washed with Sodium bicarbonate solution	56,000
Chili washed with ozone for 40 minutes	46,000

4. Removal of pesticide residues. To determine the removal of pesticide residues by ozonation time at 10 kV and air flow rate of 10 l/min, the experimental results are presented in Table 3 and Table 4. From the results showing in the tables, they can be stated that the removal of pesticide residues by gas chromatography 53 types in 20 kg of chili found 1 type of Cypermethrin. The removal of pesticide residues has been significantly affected by ozone comparing to the other treatments. This is because the ozone molecule can oxidize the pesticide residues species. Which is corresponding to the previous works (King, Szczuka, Zhang, & Mitch, 2020; Mattei, Dupont, Wortham, & Quivet, 2019; Pandiselvam *et al*., 2020). Washing 1 kg and 20 kg of chili sample with ozonation can reduce the amount of pesticide residues more than those treatments with piped water, Potassium permanganate solution and Bisodium carbonate solution (The common methods used for washing vegetables).

Table 3. Reduction of Pesticide residues on chili of 1 kg tested by MJPK test kit

Samples	Pesticide residues level
Raw chili (unwashed samples)	Not very safe
Chili washed with piped water	Not very safe
Chili washed with Potassium permanganate solution	Unsafe
Chili washed with Sodium bicarbonate solution	Unsafe
Chili washed with ozone for 10 minutes	Unsafe
Chili washed with ozone for 20 minutes	Unsafe
Chili washed with ozone for 30 minutes	Safe
Chili washed with ozone for 40 minutes	Safe

Table 4. Amount of pesticide residues reduction for chili of 20 kg tested by Gas Chromatography

Test items	Test results (piped water) , mg/kg	Test results (Sodium bicarbonate solution), mg/kg	Test results (Ozone), mg/kg	LOD, mg/kg
Organophosphate				
Methamidophos	ND	ND	ND	0.020
Mevinphos	ND	ND	ND	0.010
Diazinon	ND	ND	ND	0.010
Dicrotophos	ND	ND	ND	0.010
Monocrotophos	ND	ND	ND	0.010
Dimethoate	ND	ND	ND	0.010
Pirimiphos-methyl	ND	ND	ND	0.010
Chlorpyriphos	ND	ND	ND	0.010
Parathion-methyl	ND	ND	ND	0.010
Malathion	ND	ND	ND	0.010
Fenitrothion	ND	ND	ND	0.010
Parathion-ethyl	ND	ND	ND	0.010
Prothiophos	ND	ND	ND	0.010
Profenophos	ND	ND	ND	0.010
Triazophos	ND	ND	ND	0.010
Ethion	ND	ND	ND	0.010
EPN	ND	ND	ND	0.010
Dichlorvos	ND	ND	ND	0.010
Chlorpyriphos-methyl	ND	ND	ND	0.010

Test items	Test results (piped water), mg/kg	Test results (Sodium bicarbonate solution), mg/kg	Test results (Ozone), mg/kg	LOD, mg/kg
Pirimiphos-ethyl	ND	ND	ND	0.010
Organochlorine				
Alpha-BHC	ND	ND	ND	0.005
Beta-BHC	ND	ND	ND	0.005
Lindane (gama-BHC)	ND	ND	ND	0.005
Heptachlor	ND	ND	ND	0.005
Aldrin	ND	ND	ND	0.005
Heptachlor-Epoxide	ND	ND	ND	0.005
O,p'-DDE	ND	ND	ND	0.005
Alpha Endosulfan	ND	ND	ND	0.005
Dieldrin	ND	ND	ND	0.005
O,p'-DDD	ND	ND	ND	0.005
Endrin	ND	ND	ND	0.005
Beta Endosulfan	ND	ND	ND	0.005
Endosulfan sulfate	ND	ND	ND	0.005
P,p'-DDT	ND	ND	ND	0.005
Delta-BHC	ND	ND	ND	0.005
O,p'-DDT	ND	ND	ND	0.005
Carbamate				
Carbaryl	ND	ND	ND	0.005
Isoprocarb	ND	ND	ND	0.005
Fenobucarb	ND	ND	ND	0.005
Promecarb	ND	ND	ND	0.005
Carbofuran	ND	ND	ND	0.005
Methiocarb	ND	ND	ND	0.005
Methomyl	ND	ND	ND	0.005
Aldicarb	ND	ND	ND	0.005
Oxamyl	ND	ND	ND	0.005
Metolcarb	ND	ND	ND	0.005
Pyrethroid				
Bifenthrin	ND	ND	ND	0.005
Lamda-Cyhalothrin	ND	ND	ND	0.005

Test items	Test results (piped water), mg/kg	Test results (Sodium bicarbonate solution), mg/kg	Test results (Ozone), mg/kg	LOD, mg/kg
Permethrin	ND	ND	ND	0.005
Cyfluthrin	ND	ND	ND	0.005
Cypermethrin	0.023	0.010	<0.010	0.005
Fenvalerate	ND	ND	ND	0.005
Deltamethrin	ND	ND	ND	0.005

Note : ND means not be detected

5. Determination of specific energy consumption. To evaluate the energy consumption in the ozone generator at 10 kV, air flow rate of 10 l/min and discharge time of 0–40 min, the results are reported in Table 5. The results state that energy consumption of the ozonation system increases with discharge time. From the experiment in the last section using the 40 min ozone system will consume 0.8 kWh of electrical energy.

Table 5. Energy consumption in plasma ozoniser system

Discharge time (min)	Energy (kWh)
10	0.2
20	0.4
30	0.6
40	0.8

Conclusion

The experiment of this research work stated that ozone concentration is dependent upon voltage supply, air flow rate whilst the amount of ozone concentration can be determined by using standard KI methods. And the percentage of *E. coli* and pesticide reduction also depends on duration time of ozonation as well as ozone concentration. Ozone shows high performance for disinfection of microorganism in chili, especially on *E. coli* and pesticide.

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