Evaluation of The Effectiveness of Plasma Gel for Disinfection

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Abstract

This study was conducted to determine the effectiveness of plasma gel for disinfection and the evaluation of consumer acceptance. The efficacy of the plasma gel was evaluated based on the generating capability of hydrogen peroxide (H_2O_2) radical and the disinfection of the growth of *Escherichia coli* (*E. coli*). The plasma gel was generated using plasma-activated water (PAW) with a flyback generator. The irradiation of plasma accompanied with UV-C was also studied. Furthermore, the product's shelf-life was evaluated based on the remaining H_2O_2 concentration for 7-days. In addition, the sensory test was performed and compared with a commercial alcohol gel product. The results show that the 20 min plasma and UV-C irradiation time can induce the highest H_2O_2 in PAW, 2.92 ppm. Therefore, after plasma gel preparation at 75% concentration, the persistence of the H_2O_2 that the oxidative meantime on shelf-life of 7 days of the product was reduced from 2.19 ppm to 0.28 ppm. Additionally, the effectiveness of plasma gel for the *E. coli* reduction was 1.21 log CFU/ml. Finally, the sensory satisfaction showed that plasma gel had a higher satisfaction score than alcohol gel in terms of texture, smells, stickiness after using the product, and evaporation drying time.

Keywords
Plasma gel, Plasma-activated water (PAW), Hydrogen peroxide (H_2O_2), *Escherichia coli* (*E. coli*), UV-C

1. Introduction
Nowadays, healthcare trends are becoming very popular. Hygiene care is taken to keep them safe from bacteria such as Escherichia coli or E. coli. These gram-negative bacteria can be found everywhere and contaminate the human body. E. coli can cause food poisoning, damage to the intestinal mucosa, and potentially fatal inflammation. Eliminating E. coli on the human body could be done by keeping the parts of the body clean, especially the hands. Since hands are most likely to be contaminated with bacteria from touching the environment (Mostafidi et al. 2020). Hands can be cleaned with soap or a sanitizer containing alcohol. However, because of the COVID-19 scenario, the alcohol gel has been in short supply. This infectious disease rapidly continues to transmit from person to person, which is harmful to the respiratory system leading to death (Ceylan et al. 2020). Alcohol gel is also used to disinfect pathogens to protein, including bacteria and viruses. However, alcohol product has disadvantages for some issues such as causing dry skin and lack of moisture, and it has a strong and pungent odor. For a variety of reasons, this motivated the development of a product with disinfectant capabilities similar to those of alcohol gel while also providing moisture that does not dry out the skin, is environmentally friendly, and does not have a pungent odor. Plasma technology generally used in medicine, industry, and agriculture is an alternative approach to produce disinfection media that can substitute alcohol gel. Many previous studies show that plasma could be used for pathogen inactivation (Royintarat et al. 2020, Filipić et al. 2020, Wang and Salvi 2021, Han et al. 2020, Liu et al. 2020). This study developed the plasma system based on a non-thermal plasma system with a flyback generator and irritation UV-C. This PAW system with

UV-C can generate free radicals into the water, also known as Reactive Oxygen Species (ROS), contributing to pathogen inactivation. This ROS group consists of free radicals essential for disinfection, including H_2O_2 which help destroy bacterial cell walls and cause apoptosis (Thirumdas et al. 2018). This pathway allows protons produced by aqueous plasma beams to enter the bacterial nucleus, causing DNA malfunctions (Bhilwadikar et al. 2019).

1.1 Objectives

This research aims to evaluate the effectiveness of plasma gel in terms of free radical generation and inactivation of Escherichia coli (E. coli). In addition, shelf life and customer perception of the plasma gel product are also studied for future commercial purposes.

2. Literature Review

2.1 Plasma activated water (PAW)

PAW is a non-thermal plasma or cold plasma that generates a variety of reactive species in water to disinfect pathogens or microorganisms (Thirumdas et al. 2018). The PAW contains free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), both species also called RONS, which are capable of pathogens inactivation at the DNA (Wang and Salvi 2021). Non-thermal plasma has many applications for pathogens prevention, such as medicine, agriculture, and food. Plasma has not significantly changed physical and chemical properties and the sensory characteristics in food (Saremnezhad et al. 2021). PAW has also been used for the postharvest product to reduce bacteria and fungus, while the physical and chemical properties have not been significantly changed (Xu et al. 2016).

2.2 Free Radicals/Reactive Species

Plasma activation produces free radicals in water that can affect disinfection pathogenic microorganisms, including the ROS Group such as hydroxyl ($OH^{\cdot}$) and H_2O_2. Hydroxyl is a highly potent molecule of recombination that results in the production of H_2O_2 (Brisset et al. 2011, Thirumdas et al. 2018). Past research studied the mechanism of PAW generated by various plasma generator systems and optimized the plasma generating conditions according to the efficiency of bacterial inactivation (Royintarat et al. 2019). The study showed that plasma activation time was directly proportional to the H_2O_2 concentration and inversely proportional to the pH. In the plasma jet, H_2O_2 peaking at 1.5 ppm was able to reduce *E. coli* and *S. aureus* to 7.38 log CFU/ml and 3.06 log CFU/ml, respectively. Besides, the research studied plasma ice that can reduce Total Viable Counts (TVC) from log 6 to log 3.9 from the past study, and is caused by reactive species ROS/RNS, like H_2O_2 (Liao et al. 2018).

2.3 Mechanism $OH^{\cdot}$ /H_2O_2 on Microorganisms and Pesticide

With the mechanism of ROS generation during PAW generation, the acidic H_2O_2 species can attack the bacterial cell wall, and the $OH^{\cdot}$ penetrate the cell to cause cytoplasmic oxidation. Finally, the cell wall is breakdown and DNA is damaged. The resulting pathogenic or bacterial pathogens cannot transport food or cell activity into dead cells (Thirumdas et al. 2018), cited in (Chen et al. 2010). In addition, H_2O_2 is also used to decompose residues or harmful chemicals in the form of free radicals $OH^{\cdot}$ (Wang and Salvi. 2021). $OH^{\cdot}$ has a significant potential for reacting with other molecules in water, such as organic substances (Ghaly et al. 2001) or pesticides from the Organophosphate (OPs) group (Phunsathitwong 2019). Additionally, the research from Phan et al. (2018) employed non-thermal plasma to eliminate pesticide residues in mango. This PAW system produced by gliding arc plasma (GA) in water that induced $OH^{\cdot}$ at OES 309 nm was used to reduce chlorpyrifos and cypermethrin residues in mango that have factors consisted of Ar gas flow rates and plasma activation time, respectively. Furthermore, when compared to the control (untreated), this gliding plasma system with an Ar flow rate of 5L/min and 5-minute treatment duration was able to reduce chlorpyrifos and cypermethrin by 74.0 % and 62.9 %, respectively (Phan et al. 2018).

2.4 H_2O_2 storage

The persistence of H_2O_2 is based on a constant pH value; when the pH changes, the H_2O_2 concentration changes (Surowsky et al. 2016). The PAW's pH depends on the ambient temperature, and the half-life of H_2O_2 is typically 1 ms at the air-liquid interface. It can be considered a longevity value compared to O_2 or $OH^{\cdot}$ (Møller et al. 2007). H_2O_2 has been reported to persist in water from 8 hours to 20 days when stored out of light and in a sealed container (Surowsky et al. 2016). The amount of H_2O_2 concentration also depends on the source of the plasma (Thirumdas et al. 2018) and the duration of plasma irradiation in water. The longer time in plasma generation can lower the pH because the amount of H_2O_2 is increased due to the acidic state of H_2O_2 (Riordan et al. 2005). Moreover, the pH

change depends on temperature, resulting in higher pH values. It was also found that temperatures below 25 C° did not significantly change the pH value of PAW (Traylor et al. 2011).

2.5 Effect of plasma on *E. coli* and Virus

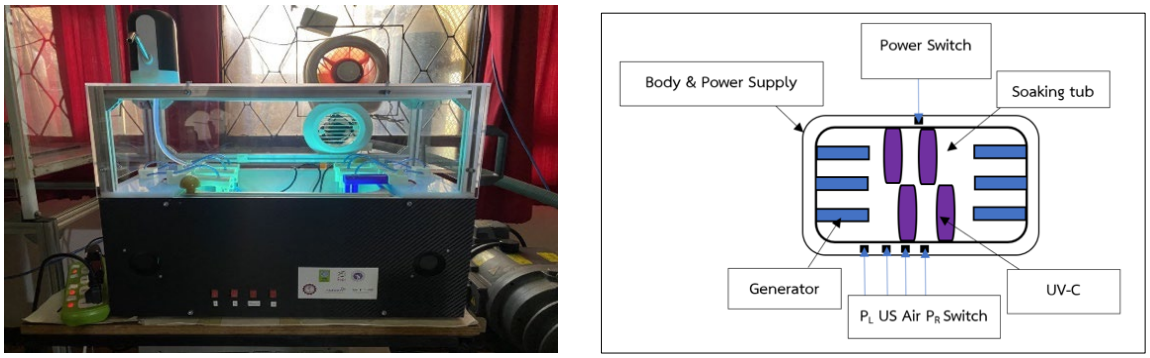
E. coli is a widespread pathogen to be tested for plasma efficacy because E. coli is a harmful bacterium or pathogen widely distributed in food and the environment. Plasma can significantly disinfect E. coli, especially with the high risk of O157:H7 strain. When E. coli gets into the body, it can cause death, like a virus attack (Han et al. 2020). The ROS Group/RONS can directly destroy nucleic acids (RNA), proteins, and lipids since viruses are made up of basic structures with lipid layers covering nucleic acids (Guo et al. 2015). With the current SARS-CoV-2 coronavirus outbreak, it's possible that RONS can cause SARS-CoV-2 coronavirus destruction. However, direct testing with the coronavirus SARS-CoV-2 is risky because it is a dangerous virus that must be researched by a medical expert. (Filipić et al. 2020).

2.6 UV-C for Radical Induction

When UV-C is combined with plasma generated in water, it helps to increase H_2O_2 production. PAW produces the free radicals of the ROS group and RNS group. RNS acts as an indirect photosensitive agent when exposed to UV-C, producing secondary oxidants such as superoxide and hydroxyl (Tarr 2003). Khienman et al. (2020) employed both PAW and UV-C for disinfection of *E. coli* in their study. They investigated 3 protocols for the *E. coli* reduction by PAW, UV-C, and PAW+UV-C, then measured H_2O_2 concentration and *E. coli* reduction ratio. The results showed that H_2O_2 from PAW, UV-C, and PAW+UV-C products were 2.09, 0, 3.34 ppm, respectively. Furthermore, the *E. coli* reduction from these conditions were 9, 10.33, 12.33 log CFU/ml, respectively (Khienman et al. 2020). Thus, UV-C exposure during plasma generation has potentially induced more H_2O_2 radicals into the water.

3. Methods

An experimental setup was employed to investigate the effect of PAW and PAW+UV-C on the effectiveness of plasma gel preparation. Figure 1 illustrates the experimental design and the layout of the Plasma and UV-C generators. In this study, H_2O_2 concentrations were measured on the PAW and PAW+UV-C with two discharge times (10 and 20 min). The H_2O_2 concentration test required a strip test indicating the color of the H_2O_2 concentration in the PAW. The H_2O_2 colorimetric measurement was employed to indicate the level of H_2O_2 concentration at different conditions of the experimental setup, as shown in Table 1. Following the experiment, the condition with the highest concentration was proposed to implement into a plasma gel.



(A) (B)

Figure 1. Plasma system with 6 flyback generators (A) Plasma+UV-C setup (B) Plasma+UV-C layout

3.1 Plasma gel preparation

Preparation of plasma gel was based on the alcohol gel formulation by the Department of Medical Sciences, Ministry of Public Health of Thailand. In general, alcohol gel contains a 75% concentration. Also, plasma gel was prepared at 75% PAW concentration. The preparation method starts from adding Carbopol 940, a gelling agent, to DI water 384 ml 4.5 g and stirring until homogeneous. Then the mixture is added with 370 ml of PAW and 1.5 g of triethanolamine

for pH adjustment. After that, plasma gel is placed into opaque storage before the H_2O_2 concentration persistence, *E. coli* reduction, and sensory tests.

3.2 Persistence of H_2O_2 testing

After preparation of plasma gel, the persistence of H_2O_2 concentration in plasma gel was tested daily for 7 days with the peroxide test strip to obtain the remaining concentrations, as shown in Table 2. Then, the obtained values were used to create equations between H_2O_2 concentrations and time using the Minitab program**.**

3.3 E. coli Analysis

For the disinfection test, the reduction of E. coli was performed using colony counting. The process started from culturing E. coli in a liquid medium and then incubated at 37 C ° for 24 h. After 24 h, the inoculum was examined for the ultraviolet of 600 nm. The absorbance value of 0.18 was required. The inoculum was placed in 4 centrifuge tubes at 1 ml per tube and centrifuged at a speed of 9000 rpm for 10 min. The test substance was injected into the control gel (without plasma water) and plasma gel. The solution was diluted 10 times by pipette from a 1 ml centrifuge tube into a test tube containing 9 ml of 0.85 % NaCl solution and diluted from 10^{-1} to 10^{-10}, and then 0.1 ml of 10^{-4} to 10^{-7} solution. Then, both solutions were spread on a plate and incubated at 37 C° for 24 hours. After inoculations, the E. coli counts were computed from equation 1. The reduction of E. coli was calculated based on the difference between E. coli counts from the control and plasma gel.

$$E.Coli\ (\mathrm{CFU/ml}) = \frac{(\text{Number of colonies} * \text{Dilution factor})}{\text{Aspirate solution}} \tag{1}$$

3.4 Sensory Evaluation

Sensory evaluation of plasma gel consisted of 6 characteristics: product texture, smell, stickiness after using the product, and evaporation drying time compared to alcohol gel products. A total of 30 volunteers was requested to rate the product's sensory score, based on a Hedonic scale of 1 to 5 (Cardello 2017). Finally, these rated scores were then plotted into a radar chart and statistically analyzed to compare the two products.

4. Results and Discussion

4.1 The results of PAW in combination with UV-C Irradiation

As illustrated in Table 1, the experimental result shows that the H_2O_2 concentration was proportional to the plasma and UV-C irradiation. Furthermore, UV-C has significantly increased the H_2O_2 concentration of PAW. As a result, the highest condition of 2.92 ppm of H_2O_2 was obtained from PAW with 20 min irradiation of both plasma and UV-C.

4.2 The Persistence of H_2O_2 Test

Table 2 shows the H_2O_2 concentration in the plasma gel tested daily for 7 days. The result is illustrated in graphical format in Figure 2. As can be seen, the concentration was exponentially decreased from day to the end of day 7. On days 1-3, the concentration of H_2O_2 decreased from 2.19, 1.47. and 0.98 respectively, with a supine parabolic graph. From day 3 to day 6, the concentration of H_2O_2 was evenly reduced linearly before exponentially decreasing on day 7. This is demonstrated by a regression model with an R-Sq (adj) of 99.8%. The gradual decrease of H_2O_2 concentration in plasma gel is based on the half-life of the radical, which allows it to disintegrate back into water and air for a short period of time (Brisset and Pawlat 2015). Furthermore, many factors affect the persistence of H_2O_2 concentrations depending on pH and temperature. A previous study shows that the persistence of H_2O_2 concentration decreased when pH and temperature were increasing (Zhou et al. 2019). Another study by (Liao et al. 2018) applied PAW to generate plasma ice for shrimp preservation. The results showed that plasma ice could maintain the freshness of shrimp over 9 days in a stomacher bag at (25 ± 1 C°). The infection increased on day 7 by log 5 from log 3.9 compared to day 1 when pH increased, indicating a gradual degradation of ROS free radicals changing related to the environment, temperature, and pH.

4.3 The E. Coli Reduction Test

The spread plate method performed an efficacy test for the disinfection of *E. coli* (TISTR 117). The results show that the control gel at dilution levels 10^{-6} contained colonies on the culture plate, as shown in Figure. 3(A), while the plasma gel at 10^{-6} dilution contained a smaller number of colonies on the plate, as shown in Figure 3(B). Table 3

displays the computed findings of the number of bacteria (log CFU/ml), which shows that PAW from plasma and UV-C irradiation reduced the number of *E. coli* by 1.21 log over the control gel.

4.4 The Sensory Evaluation Between Alcohol Gel and Plasma Gel

Figure 4 illustrates the plasma and alcohol gel satisfaction results on 30 volunteers. When 6 characteristics were tested comprising of texture, smell, dryness after using, stickiness after using, moisture after using, and evaporation drying time. Also, it was found that plasma gel had higher satisfaction scores than alcohol gel in terms of characteristics consisting of texture, smell, stickiness, and the evaporation drying time (p-value < 0.05).

5. Results and Discussion

5.1 Numerical Results

Table 1. H_2O_2 concentration of PAW

(min)	H_2O_2 concentration (ppm)	
	Plasma	Plasma + UV-C
10	1.43	1.69
20	2.36	2.92

Table 2**.** The persistence of hydrogen peroxide (H_2O_2)

Days	H_2O_2 concentration (ppm)	Temperature (C°)	Reduction of H_2O_2 concentration compared with Day 1 (%)
1	2.19	30	0
2	1.47	28	33
3	0.98	27	55
4	0.83	26	62
5	0.69	26	68
6	0.55	26	75
7	0.28	27	87

Table 3. The number of *E. coli* on the control gel and plasma gel plate

Plate	Control (log CFU/ml)	Plasma (log CFU/ml)	Reduction (log CFU/ml)
1	8.81	7.58	1.23
2	8.95	7.70	1.25
3	8.86	7.72	1.14
Average	8.87 ± 0.07	7.66 ± 0.07	1.21

5.2 Graphical Results

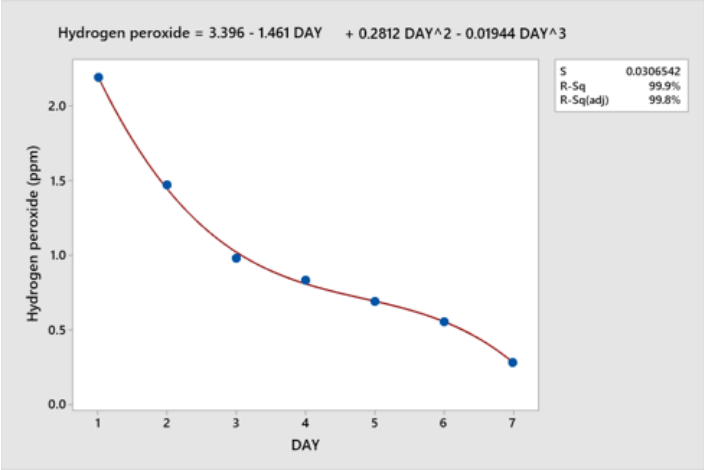


Figure 2. The reduction of H_2O_2 concentration with cubic graph

(A) (B)

Figure 3. The number of *E. coli* colonies on (A) control gel plate (B) Plasma gel plate

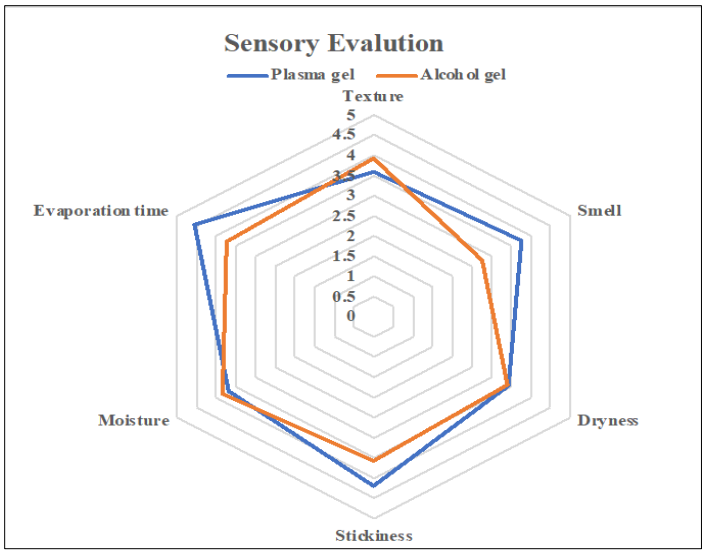


Figure 4. Sensory evaluation with plasma gel and alcohol gel

5.3 Proposed Improvements

There are still limitations on skin irritation testing and COVID-19 virus testing for this research. Especially the virus test can be performed only in advanced medical laboratories. In addition, it also has to test the sustainability of the product to the actual use of the consumer. This could ensure that plasma gel products can be an alternative disinfection product for consumers.

6. Conclusion

This study investigated the possibility of using PAW in gel formulation as alternative disinfection. The first experimental setup studied the effect of UV-C on PAW generation and the irradiation time. The result shows that PAW with UV-C irradiation at 20 min exposure time can induce the highest H_2O_2, 2.92 ppm. Then, similarly to alcohol gel for disinfection, plasma gel was prepared using 75% concentration. Then, the efficacy test of plasma gel in 3 issues was performed: the persistence test of H_2O_2 concentration in 7 days, the effective reduction of *E. coli* test, and the sensory satisfaction test. According to the results of the persistence test, after 7 days, the H_2O_2 concentration in the plasma gel decreased from 2.19 to 0.28 ppm. Next, the spread plate method investigated the efficacy test for reducing *E. coli* (TISTR 117). The ability of the control gel and the plasma gel to reduce *E. coli* was compared in this experiment. According to the findings, plasma gel products can reduce bacterial infection by more than 1.21 log CFU/ml, making them suitable for disinfection. The final test is the sensory comparison between plasma gel and alcohol gel with 6 characteristics: texture, smell, dryness, stickiness, moisture, and evaporation drying time. According to 30 participants, consumers prefer plasma gel to alcohol gel in terms of smell, stickiness, and evaporation drying time. Further study to optimize PAW condition and effective virus evaluation are among project milestones to develop an alternative disinfection substance for the consumer.

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Biography

Soithong Lungparn is currently a full-time assistant researcher in Advanced Manufacturing and Management Technology Research Center (AM2Tech), Department of Industrial Engineering, Faculty of Engineering, Chaing Mai Univerity. She holds a Bachelor of Science degree in Food Processing Engineering from Chaing Mai University and a Master of Engineering degree in Industrial Engineering from the same university. Her research focuses on applying PAW with UV-C and Ultrasound to reduce pesticide residues in agricultural products.

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