1. INTRODUCTION

1.1. Background

Strawberry is one of economically high-valued fruit, yet it is vulnerable to bacterial and fungal contamination. The shelf life of the fruit remains short despite being stored in the refrigerator (Issa-Zachari *et al*, 2010). Post-harvest washing, which is required on the handling of most fruits, is not recommended in strawberries due to the acceleration of *Botrytis cinerea* growth. In general, the surface of strawberries could contain pathogenic microorganisms which pose danger to consumers. An outbreak of hepatitis A disease once occurred due to contamination of commercially produced frozen strawberries (Flessa *et al*, 2004).

Microorganisms that are commonly found on the surface of fresh strawberries are *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. These microorganisms remain dormant on low temperature storage (Flessa *et al*, 2004). Chlorine is commonly used to reduce microbial count on fruits and vegetables during post-harvest management. However, the application of chlorine on strawberries only produces 1-2 log reduction yet increasing the concentration is not recommended as it might raise environmental and health problems (Udompijitkul *et al*, 2007).

Electrolyzed water is a result of electrolysis process. The application of electrolyzed water could reduce up to 7 log of microbial log on tomatoes, 4 log on spinaches and 1-3 log on other fruits and vegetables (Rahman, 2016). Electrolyzed water could also act as a surface disinfectant for glass, stainless steel and ceramic materials (Park *et al*, 2002). The most apparent advantage of electrolyzed water application is worker safety as the product poses no threat for human. The production, distribution, handling, storage and application are simpler compared to other disinfectants such as sodium hypochlorite and chlorine which could be dangerous. Despite the simplicity, the product contains profound antimicrobial performance and does not alter the appearance of treated samples. Moreover, electrolyzed water production is enviromentally friendly as there were zero heat utilization and the product contains bactericidal, fungicidal, and virucidal properties (Udompijitkul *et al*, 2007). Electrolyzed water bactericidal properties could be enhanced with agitation during the application. Agitation could reduce the amount of chlorine residues on electrolyzed water after the treatment by 5 times, suggesting that the application increases the contact between chlorine residues and microorganisms on samples. Previous studies had not researched the microbial population reduction of dipping and agitating strawberries in electrolyzed water. The efficacy of the hurdle was researched in this study.

1.2. Literature Review

1.2.1. Strawberry Characteristics

Strawberries are one of the most expensive crops in Indonesia, right after pepper and chillies (BPS Tabanan, 2016). The cultivation productivity (ton/ha) of the fruit is high, reaching up to 200 ton per hectare. However, the land allocated for the cultivation of strawberries and the production of the fruit are one of the lowest among other fruits (0.3% of total fruit production in Indonesia).

Table 1. Strawberry Production in Indonesia from 2009-2014

Year	Cultivation Area (ha)	Yield (ton)	Average Yield (ton/ha)
2009	840	19,132	22.78
2010	1159	24,846	21.44
2011	987	41,035	41.58
2012	810	169,796	209.62
2013	745	90,352	121.28
2014	787	58,882	74.82

(Direktorat Jenderal Hortikultura, 2015)

Table 1 shows the cultivation area, total yield and average yield of strawberry production in Indonesia. As seen on the table, cultivation area and yield of strawberries are not directly proportional. Strawberries are not widely cultivated due to the growth temperature requirement. Ripe strawberries are also highly perishable, therefore the amount of loss are sizeable. The cause of the problem is the physical structure of strawberries. As the surface of the fruit contains seeds, the area become uneven and provides ideal shelter for microorganisms. Before harvesting time, strawberries tend to rapidly ripen which causes the cell walls to soften in a short period of time. The cell walls continue to soften after harvesting time as the fruit respirate and the process could not be suppressed by storing the fruit in low temperature. The soluble contents of ripe strawberries consist of sugar such as glucose, fructose and sucrose (Li *et al*, 2014). The softening of cell walls and the rapid surge of sugar content increases the susceptibility of the fruit to microorganisms. As a result, the fruit deteriorates quickly even in low temperature storage (Woodward, 1972).

Microorganisms commonly found on strawberries are *Botrytis cinerea, Listeria monocytogenes* and *Escherichia coli*. *Escherichia coli* and *Listeria monocytogenes* could be found on fruits due to cross contaminations from animals, irrigation water and soil. Splashes of soils due to irrigation or rainfall helps the bacteria to travel to the plant. Water also eases the movement of bacteria to the roots of the plant. A few outbreaks occurred due to *E. coli* contamination on strawberries (Shaw *et al*, 2015).

Botrytis cinerea could be found on the surface of strawberries due to contamination from the soil and airborne spores. The spores start to germinate in drops or films of water. The spores could germinate up to 90% after 4 hours of contact with water drop or water film. Although water films generally do not last long enough to cause post-germination infection, the infection could occur when water films are trapped on parts of the plant. Infection of *Botrytis cinerea* on strawberries could cause over 50% of post-harvest loss (Jarvis, 1961).

1.2.2. Characteristics of Contaminant Microorganisms

Escherichia coli and *Liesteria monocytogenes*, which are commonly found on the surface of strawberries, are highly vulnerable to electrolyzed water. Electrolyzed water which contains 0.2 to 0.5 mg/l chlorine residue could produce 5-6 microbial log reduction, while 1 mg/l chlorine residue could eliminate both population (Park *et al*, 2003). According to Venkitanerayanan *et al* (1999), dipping microorganisms on electrolyzed water for 5 minutes could reduce the population to less than one log. Meanwhile dipping the microorganisms for 10 minutes could eliminate the population. On 35^{o}C electrolyzed water, less than one log of *Salmonella enteritidis* remains while *Escherichia coli* and *Listeria monocytogenes* were eliminated after 2 minutes of dipping. On 45^{o}C electrolyzed water, the population of *Salmonella enteritidis* and *Listeria monocytogenes* were reduced by 7 log while *Escherichia coli* were completely eliminated after 1 minute of dipping (Venkitanerayanan *et al*, 1999).

The resistance of microorganisms in electrolyzed water depends on its growth phase, cell wall thickness and pH of the product. The resistance of *Escherichia coli* and pH increment is directly proportional, therefore the bacteria tend to be highly resistant to alkaline electrolyzed water, slightly resistant to slight acidic electrolyzed water, and not resistant in acidic electrolyzed water (Rahman *et al*, 2016). Bacteria spores tend to be more resistant than vegetative cells (Rahman *et al*, 2012). The thickness of microorganisms' cell walls and resistance on electrolyzed water is directly proportional. *Botrytis cinerea,* which could be found on fresh strawberries, could be completely eliminated after 30 seconds of dipping in electrolyzed water due to its thin cell walls (Buck *et al*, 2002).

1.2.3. Sensory Qualities

Dipping strawberries in electrolyzed water produces no defect in appearance and no significant difference in pH, sugar and acid content (Hung *et al*, 2010b). However, the shelf life of treated and untreated strawberries were not significantly different as well (Hung *et al*, 2010a). In a study conducted by Jeong *et al* (2006), dipping strawberries in low alkaline electrolyzed water reduces the color of the fruit, yet increases its firmness, thus increasing the sensory qualities. In another study conducted by Ding *et al* (2015), hurdle application of slightly acidic electrolyzed water and ultrasonification produces no significant difference in vitamin C, antocyanin, acid and solid content. The treatments also produces no difference in firmness and other sensory attributes.

1.2.4. Characteristics of Electrolyzed Water

The production of electrolyzed water commonly requires 10-20 V of electrical current. On less than 10 V, the product contains less oxidation-reduction potential, chlorine residues, and higher pH. Therefore, the bactericidal properties are weak and less efficient (Hung *et al*, 2010b). The bactericidal properties of electrolyzed water could be enhanced by increasing the electrolyzing time. Increasing electrolyzing time causes pH reduction and increases oxidation-reduction potential of the product (Rahman *et al*, 2012).

Generally, tap water or groundwater are used to produce electrolyzed water. Groundwater naturally contains ions such as sodium (Na), magnesium (Mg), calcium (Ca), chloride (Cl), potassium (K) and iron (Fe) (Krisna, 2011). During electrolysis, positively charged ions move towards the cathode, forming the acidic electrolyzed water. Meanwhile, negatively charged ions move towards the anode, forming the alkaline electrolyzed water. During electrolysis, the ions also react with dissolved oxygen and hydrogen ions in the groundwater, forming HOCl or OCl^-. The electrolysis process is depicted in Figure 1.

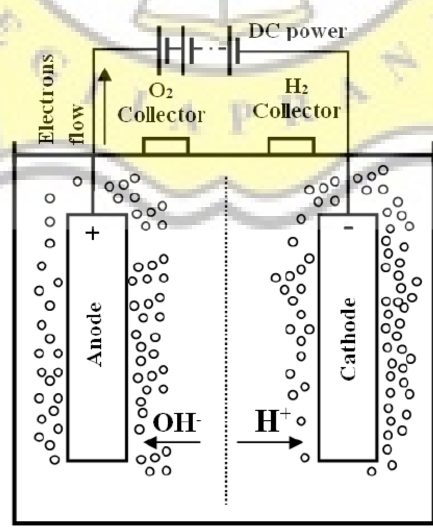

Figure 1. Electrolysis Process Scheme (Azmy, 2015)

Oxidation-reduction potential (ORP) could be used to measure the antimicrobial properties of electrolyzed water. ORP value is directly proportional to the amount of free electrons and inversely proportional to pH (James *et al*, 2004). On solutions which contain 1 mg/l of chlorine, the ORP value could reach 900 mV. On 2 mg/l of chlorine concentration, the ORP value could reach 950 mV and on 5 mg/l of chlorine, the ORP value could reach 1000 mV. These values could be achieved as chlorine is a strong oxidant. Oxidants in electrolyzed water could oxidize sulfhdryl compounds on cell surfaces and other key metabolic systems which inhibites bacteria growth and evetually eliminate the bacteria (Park *et al*, 2003). Most bacteria and viruses are eliminated at ORP value of 600 mV (Maharjan *et al*, 2016).

The storage condition of electrolyzed water influences its bactericidal properties. During storage, decomposition of chlorine concentration occurs and reduces the oxidation-reduction potential of electrolyzed water. Storing the product in an open container further reduces the power due to evaporation of chlorine residues and pH increment, therefore reducing the bactericidal properties of the product (Len *et al*, 2002). Product stored in an open container could be utilized to inactivate *Escherichia coli* and *Listeria monocytogenes* up to 6 days after electrolyzation. Meanwhile, products could be utilized up to 14 days after electrolyzation if stored on a closed container (Rahman *et al*, 2012). The bactericidal power of product could be reduced further by agitation and and storage temperature. Agitating product could reduce the power by 5 times (Len *et al*, 2002). After 24 hours of storage, product stored in 4^{o}C maintains its bactericidal properties as chlorine residues settled while product stored on 25^{o}C starts to lose its chlorine residues and bactericidal properties (Fabrizio and Cutter, 2003). However, inactivation of microorganisms were significantly more effective on higher temperature (Rahman *et al*, 2016).

1.2.5. Influence of pH

Electrolyzed water could be categorized based on its pH: strong acid electrolyzed water (pH ≤2.7), slightly acidic electrolyzed water (pH 5-6.5), neutral electrolyzed water (pH 6.5-7.5) and alkaline electrolyzed water (pH 10-13). Anodes and catodes highly influence the pH of electrolyzed water. Anodes produce high chlorine residue, high oxidation-reduction potential and low pH while catodes produce the opposite results (Koseki *et al*, 2003).

Table 2. pH Influence on Microorganisms Population Reduction

Treatment	Microorganism reduction (log)		
	Aerobic mesophillic bacteria	*Salmonella* spp	*Eschericia coli*
Strong acid electrolyzed water (pH ≤2.7)	2.07 ± 0.03	2.22 ± 0.02	2.77 ± 0.02
Slightly acidic electrolyzed water (pH 5.6)	1.68 ± 0.04	2.12 ± 0.03	2.21 ± 0.05

(Issa-Zachari *et al*, 2010)

Koseki *et al* (2003) studied the population reduction of microorganisms on strawberries treated by 10 minutes of dipping in strong electrolyzed water (StEW) and 5 minutes of dipping in StEW followed by 5 minutes of dipping in alkaline electrolyzed water (5+5). The results of the study is listed in Table 2.

Table 3. Microorganisms Population Reduction under Different pH and Dipping Time

Treatment	Microorganism population (log)		
	Aerobic mesophillic bacteria	Coliform bacteria	Fungi
None	4.8 ± 0.2	2.4 ± 0.5	3.7 ± 0.4
Tap water	4.6 ± 0.4	2.1 ± 0.7	3.6 ± 0.4
StEW (pH 2.6)	3.2 ± 0.2	< 1.3	2.0 ± 0.6
5+5 (pH 2.6 and 11.3)	3.7 ± 0.3	< 1.3	2.9 ± 0.9

(Koseki *et al*, 2003)

When the pH of electrolyzed water is higher than 7.5, chlorine residues could be found as hypochlorite ions (OCl^-) in a small amount (Rahman *et al*, 2010). In acidic electrolyzed water, chlorine residues could be found as hypochlorous acid (HOCl). The former eliminate microorganisms by oxidizing and inactivating proteins on cell plasm through the outside of the cell (Fukuzaki, 2006). Meanwhile, the later has more bactericidal properties as HOCl has the ability to break fat tissues and microorganisms' cell membranes. The difference in bactericidal power is up to 80 times (Udompijitkul *et al*, 2007). In a study conducted by Jeong *et al* (2006), dipping strawberries in low alkaline electrolyzed water (pH 8) for 20 minutes merely reduces 2 logs of the microorganisms populations. Dipping the fruit in electrolyzed water with lower pH and in a shorter amount of time produces identical results. According to Rahman *et al* (2010), on an even higher pH (≥9), electrolyzed water is unable to eliminate microorganisms. The main differences of acidic and alkaline electrolyzed water are listed in the following table.

Table 4. Characteristics of Acidic and Alkaline Electrolyzed Water

Factors	Acidic Electrolyzed Water	Alkaline Electrolyzed Water
pH	≤ 2.7 (strong acid) 5 - 6.5 (slightly acidic)	Alkaline (10-13)
Ions	Negatively charged	Positively charged
Free Chlorine Form	HOCl	OCl^-

1.2.6. Influence of Time

Dipping time of strawberries highly influences the penetration of electrolyzed water due to its uneven surface. In a study conducted by Ding *et al* (2014), dipping strawberries in slightly acidic electrolized water for 5 and 10 minutes produces insignificant result of merely 0.5 to 1.5 log reduction of aerobic mesophillic bacteria, coliform bacteria, and fungi. As suggested by Koseki *et al* (2003), 10 minutes of dipping time in strong acid electrolyzed water could effectively reduce microorganisms populations, which is 1-2 logs of aerobic mesophillic bacteria, coliform bacteria, and fungi. Another study conducted by Xu *et al* (2013) showed that 15 minutes of dipping in strong acid electrolyzed water or slightly acidic electrolyzed water reduces 94.8% bacteria population on the surface of strawberries. The reduction showed in the study proved that the application of slightly acidic electrolyzed water produces no significant difference compared to sodium hypochlorite (NaClO) application despite containing half the amount of chlorine residues.

1.2.7. Influence of Further Treatment

The effectiveness of electrolyzed water bactericidal properties could be enhanced by agitating the samples during the application. Agitation increases the distribution of chlorine residues and physically removes microorganisms cells on fruits' surface. Removing microorganisms cells from the fruit surface reduces the microorganisms' resistance and eases inactivation (Park *et al*, 2002). Up to 5 times of chlorine residues in electrolyzed water could be reduced when agitation is applied as more residues contacted the microorganisms cells (Len *et al*, 2002). The uneven surface of strawberries causes hindrance on its microbial load reduction. As increasing the contact between the surface of the fruit and electrolyzed water could increase the efficacy of microbial elimination, combining dipping and agitation of fruits in electrolyzed water could possibly reduce the microbial load.

1.3. Research Objective

The objective of the preliminary study was to determine the production time, shelf life and antimicrobial performance of electrolyzed water. The aim of the main study was to examine the microbial load reduction on the surface of strawberries by dipping and agitation in electrolyzed water with acidic and alkaline pH. The main study measures the microbial load reduction by profiling bacterial DNA on agarose gel and identifies fungi found on strawberries after storage.