4. DISCUSSION

4.1. Preliminary Study

4.1.1. Production Time and Shelf Life of Electrolyzed Water

Electrolyzed water bactericidal properties are influenced by a few characteristics, namely pH, chlorine residues, and oxidation-reduction potential. On pH 2.5 to 5, chlorine residues are found in the form of volatile chlorine gas and hypochlorous acid (HOCl) which were formed by hydrolysing electrochemically produced chlorine gas using the following formula:

$$Cl_2 + H_2O \rightleftarrows HOCl + H^+ + Cl^-$$

On pH > 5, chlorine residues are found in the form of OCl^-. The amount of OCl^- is directly proportional to the increment of pH (Park *et al,* 2003). Besides OCl^-, positively charged ions move towards the cathode to form alkaline electrolyzed water. Positively charged ions that could be found on groundwater are calcium, sodium, potassium, iron and magnesium. Meanwhile, negatively charged ions such as chloride, iodine, sulphur and phosphorus move towards the anode to form acidic electrolyzed water. The amount of available ions influences the pH of the products and the production time. The higher the concentration of negatively charged ions available, the lower the pH of acidic electrolyzed water formed. The higher the concentration of positively charged ions available, the higher the pH of alkaline electrolyzed water formed. For both products, the concentrations of available ions are inversely proportional to the production time. Production time could be lengthened to form the lowest or highest pH of products possible with the amount of available ions (Rahman *et al*, 2012). In this study, groundwater requires 6 days to be able to reach the lowest pH (3.5) possible using the available ions in the groundwater itself.

As seen on Figure 5, the pH value of the tap water used to produce acidic electrolyzed water decreased sharply during the first 3 days and finally reached 3.5 after 6 days. Meanwhile, the oxidation-reduction potential steadily increased until the 6th day. ORP could indicate the availability of free electrons in the product. ORP value tends to be significantly high in the presence of chlorine. ORP value > 600 mV in acidic electrolyzed water indicates that the amount of free chlorine available is enough to inactivate coliform bacteria and viruses (James *et al*, 2004). Meawhile, Figure 6 displayed the pH and oxidation-reduction potential during the production of alkaline electrolyzed water. The pH increased steadily while the oxidation-reduction potential tend to decline. The results are consmistent with a study conducted by James *et al* (2004) in which the ORP value of water tends to decrease with increasing pH. Therefore, alkaline electrolyzed water does not contain enough free chlorine to inactivate coliform bacteria.

During storage, the chlorine residues of electrolized water gradually reduce due to the evaporation and self-decomposition of HOCl. The pH of electrolyzed water highly influences the rate of chlorine loss. On pH 2.5 to 4, H^+ decreases and forms HOCl with the increase of pH. As HOCl is not volatile, the chlorine evaporation rate is lower with the increase of pH. The amount of chlorine residues is directly proportional to the oxidation-reduction potential of electrolyzed water. (Len *et al*, 2002).

As the samples were stored in the refrigerator, the reduction of quality was relatively slow. The results obtained were in accordance with a study conducted by Fabrizio and Cutter (2003) in which the pH and oxidation-reduction potential of electrolyzed water were more stable in 4°C in comparison with those stored in 25°C. In 25°C, the free chlorine of acidic electrolyzed water was rapidly deteriorated after 3 days. In this study, the shelf life was determined by calculating according to the regression equation obtained from 7 days of observation. The acidic electrolyzed water is considered no longer effective once the oxidation-reduction potential is lower than 600 mV. On oxidation-reduction potential lower than 600 mV, electrolyzed water no longer has the ability to inhibit the growth of microorganisms. Alkaline electrolyzed water is considered no longer effective once the pH reached 8. On pH lower than 8, electrolyzed water is considered neutral and no longer alkaline.

4.1.2. Antimicrobial Performance

The low pH of acidic electrolyzed water is believed to be able to reduce bacterial growth by sensitizing the outer membrane of bacterial cells and eases the entry of

HOCl into the cell. HOCl disrupts the cytoplasmic enzymes and membranes functions, thus inactivating the bacteria. The synergy between pH and chlorine residues is effective when the amount of chlorine residues is lower than 1 mg/l. If the amount of chlorine residues available is higher than 2 mg/l, the inactivation proceeds regardless of the pH. Therefore, the amount of chlorine residues in the electrolyzed water is crucial to the inactivation of bacteria (Park *et al*, 2003).

In another study conducted by Koseki& Itoh (2000), the amount of available chlorine concentration is no longer relevant to the bactericidal power of acidic electrolyzed water on pH 2.4 or lower. Moreover, the study concluded that tap water that had been adjusted to pH 2.4 is as bactericidal as acidic electrolyzed water. However, the available chlorine concentration and the bactericidal performance is directly proporsional. On pH 2.4 and 0 ppm chlorine concentration, bactericidal effect was present although weaker than samples with higher chlorine concentration. It could be concluded that low pH and high chlorine concentration are equally needed to produce the optimum bactericidal result.

HOCl comprises 97% of chlorine residues in acidic electrolyzed water. HOCl has the potential to inactivate bacteria. However, the bactericidal properties are highly influenced by the presence of organic matter. In an environment in which the bacteria are given an advantaging condition to grow, the bactericidal performance decreases. The bactericidal performance could be significantly reduced with the presence of proteins (Kiura *et al*, 2001). Meanwhile, alkaline electrolyzed water contains OCl^-. OCl^- is up to 80 times weaker than HOCl in terms of bactericidal properties, therefore contributing to the inability of alkaline electrolyzed water in forming inhibition zone (Udompijitkul *et al*, 2007). The study results were in line with the statement. As seen on Table 5, the disinfecting power of acidic electrolyzed water is higher than alkaline electrolyzed water. In acidic electrolyzed water samples, the disinfecting power decreased with time as the pH increased. In alkaline electrolyzed samples, there was no disinfecting power available. The increment of dissolved oxygen as seen on Table 6 also shows the breakdown of chlorine residues from HOCl and OCl^- overtime which releases oxygen particles into the electrolyzed water samples.

4.2. Main Study

4.2.1. Physical Appearance

As concluded by previous studies, dipping strawberries in electrolyzed water produces no defect in physicochemical properties. The appearances of the fruits were no different than those dipped in tap water. According to Jeong *et al* (2006), dipping strawberries in electrolyzed water increases the firmness of the fruit. The observation result shows that the samples treated with acidic electrolyzed water did not show any signs of cracking or shrivelling for 7 days. The cracks on strawberries were formed as a result of moisture content loss. The loss of moisture content reduces the fruits' turgidity, causing the fruit skin to be less flexible and shrivelled (Nunes *et al*, 1998). It could be deduced that in samples treated with acidic electrolyzed water, the rigidity of the cell walls increased. The increment of cell walls' rigidity retards the loss of moisture content, causes the product to stay firm and fresh for 7 days. The increment in rigidity could be caused by phosphorus ions in acidic electrolyzed water. In a low amount, phosphorus could increase the total soluble sugar, Brix degrees and firmness of strawberries. Therefore, phosphorus is often incorporated in fertilizers (Estrada-Ortiz *et al*, 2012). Samples treated with alkaline electrolyzed water, tap water, chlorine, and H_2O_2 showed signs of cracking or deformation after 3 days of storage.

Samples treated with tap water were overgrown by fungi after 7 days of storage. Even though the samples were stored in the refrigerator, the temperature is insufficient to completely inhibit the growth of fungi. As the surface of strawberriesis uneven, there are areas that could not be reached by the disinfectants. Thus, microorganisms that reside in the area stayed on the surface of the fruit. On foodstuffs with an even surface, tap water could prolong shelf life by removing microorganisms from the surface. However, tap water does not contain disinfecting properties as much as the other solutions utilized in the study. Therefore, dipping strawberries in tap water did not prevent the growth of fungi.

Samples treated with H_2O_2 were discolorated right after the treatment. Hydrogen peroxide has strong oxidizing properties and is commonly used as bleaching agent in industries. Treatment in food industries is limited to light-colored products such as fresh-cut fruits, fish, soybeans, and noodles. Strawberries contain anthocyanin which produces the color of the fruit. Anthocyanin could be quickly oxidized by hydrogen peroxide in room temperature by converting its malvin chloride into malvone with up to 65% yield. The conversion causes the product to be heavily discoloured (Sondheimer & Kertesz, 1951).

4.2.2. Gel Electrophoresis

Figure 10 shows that dipping strawberries in any solutions could remove microorganisms cells from the surface of the fruits. However, repeating the treatment for the 2nd and 3rd time did not remove more cells, except for the samples treated with acidic electrolyzed water. The removement of cells is indicated by the visibility of bands on the gel. As seen on Figure 10, dipping samples in chlorine and alkaline electrolyzed water removed more cells than the other solutions. The bands are less visible in samples treated with H_2O_2.

Figure 11 shows the result of DNA electrophoresis of samples that were dipped and agitated in cleaning solutions. The bands of samples treated with tap water are the most visible while those treated with alkaline electrolyzed water are the least visible. Repeating the treatment for the 2nd time removed more cells on samples treated with chlorine and acidic electrolyzed water.

Overall, treating samples with acidic electrolyzed water removed more microorganisms' cells than other solutions. The utilization of shaker to agitate the samples also increased the amount of cells removed. The results are in accordance with previous studies conducted by Park *et al* (2002) and Len *et al* (2002). Park *et al* (2002) stated that agitation could increase the distribution of chlorine residues and physically removes microorganisms' cells from the surface of the fruit. Len *et al* (2002) stated that as much as 5 times of chlorine residues could be reduced from the solutions when agitation is applied. The study confirms that the application of agitation indeed removed more cells compared to merely dipping the samples.

4.2.3. Fungal Identification

There were 3 types of fungi found on samples, namely *Aspergillus* sp, *Botrytis* sp and *Rhizopus* sp. The sample with the shortest shelf life was the samples dipped and agitated in tap water in which fungi grew on samples after 7 days. On tap water and alkaline electrolyzed water samples, all 3 fungi genera were found. *Aspergillus* sp and *Rhizopus* sp were found on acidic electrolyzed water and H_2O_2 samples. The only fungi genera found on chlorine samples were *Rhizopus* sp.

The *Aspergillus* species were mainly identified by their thin stipe, followed by its swelled apex, and present conidia head. The conidia head consists of the apex itself and phialides or metualae that are attached directly to it. The conidia shape ranges from columnar to radiate. *Aspergillus* sp ranges in color from white, green, to brown. On food products, *Aspergillus* sp can be identified by their fibrous appearance. *Botrytis* sp ranges from light grey to dark brown. The conidiophores are branched in the apical region. The colonies tend to be broad, although some could be found in solitude as well. *Rhizopus* species were identified by the presence of rhizoids. The color of the genera grows from white to greyish-black as the sporangiosphores turn brown while the fungi ages. *Rhizopus* sp are commonly found in warm areas and could grow on various foodstuffs, ranging from grains and nuts to fruits and vegetables.

Flessa (2004) stated that *Botrytis* sp are commonly found in strawberries. In this study, only 2 samples out of 30 were overgrown by the fungi. *Aspergillus* sp were found on 8 samples and *Rhizopus* sp were found on 25 samples. According to Harris & Dennis (1980), *Rhizopus* sp are a lot more common on stored strawberries as the number of species that could overgrow the fruit is higher and the humidity requirement is lower than other genus. According to Abdelfattah *et al* (2016), the abundance of *Aspergillus* sp are relatively small.

As seen on Table 8, the repetition of treatment did not significantly retards the growth of fungi on samples treated in tap water and alkaline electrolyzed water. The identification result is in accordance with the electrophoresis result. As the repetition of treatment did not significantly remove more cells, the growth of fungi on samples that were treated repeatedly was not significantly different either. Repetitions were effective to remove more bacterial cells and retard the growth of fungi for samples treated with acidic electrolyzed water, chlorine and H_2O_2.

However, the growth of fungi was preceded by the formation of defects or the decrement of appearance. On tap water samples, the samples were no longer fresh after 3 days and fungal colonies appear after 7 days. On acidic electrolyzed water, the samples were fresh up to 7 days while the fungal colonies grew after 13 days. These results were achieved after treating the samples once; therefore, no repetitions were necessary.

The result shown in Table 8 also shows that agitating samples does not significantly prolong the shelf life of the fruits. The growth of fungi on samples shows that although a lot more microorganisms' cells were removed by agitating, there are cells that were left on the surface of the fruit. Physical stress on the agitated samples could be a factor that encouraged the growth of fungi.

The growth rate and the genera of the fungi that grew on samples were highly influenced by the types of disinfectant. On tap water and akaline electrolyzed water samples, all 3 fungi genera were found which indicates that alkaline electrolyzed water samples did not provide any disinfecting properties. Despite being the most commonly found fungus in strawberries, *Botrytis* sp was not found on samples treated with chlorine, H_2O_2 and acidic electrolyzed water. Chlorine provides disinfecting properties against *Botrytis* sp and *Aspergillus* sp. Previous studies concluded that *Botrytis* sp are vulnerable to chlorine while *Rhizopus* sp are less vulnerable (Avis *et al*, 2006). *Botrytis* sp was not found on any samples treated with H_2O_2 and the samples were mostly overgrown by *Rhizopus* sp. According to Gil-ad & Meyer (1999), *Botrytis cinerea* has the ability to break down hydrogen peroxide. However, on concentration higher than 0.27 mg/l, hydrogen peroxide could eliminate 99% of *Botrytis* spores. In samples treated with acidic electrolyzed water, no samples were overgrown by *Botrytis* sp. According to Buck *et al* (2002), acidic electrolyzed water could eliminate thin-walled fungi such as *Botrytis* sp and *Monilinia* sp. The study also concluded that mixing tap water with electrolyzed water reduced the bactericidal power of electrolyzed water.