4. DISCUSSION

4.1. Washing Solutions Characteristics

This study used four types of washing solutions i.e. tap water, 100 ppm chlorinated water, AEW, and BEW. Tap water used in this study met the main Permenkes 416/Men.Kes/Per./IX/1990 i.e colourless, odourless, and tasteless and has neutral pH. The concentration of chlorinated water was adjusted to 100 ppm because it is the minimum concentration for using chlorine in agricultural industry based on Okull & Laborde (2004). The basic characteristics of washing solutions were measured including ORP, pH, and DO. The ORP value may be the major factor of antimicrobial activity of washing solutions. The value of ORP indicates the oxidation and reduction capability of solution. Based on the result, the highest ORP was found in AEW and the lowest was in BEW. According to Park *et al*. (2004), high ORP is associated with the strong bacterial activity. The strong oxidation ability of electrolyzed water might cause the oxidation of sulfhydryl compounds on cell surfaces of microorganisms and disturb the metabolic systems then the growth of microorganism cells are inhibited (Kim *et al*., 2000).

As seen on Table 4, in Electrolyzed Water (EW) samples, when the ORP value was high, the pH value was low. The results were in accordance with Park *et al*. (2004) who stated that the values of ORP and pH are inversely proportional. The pH also influences the antimicrobial activity of EW. When the pH decreases, the antimicrobial activity will increase. Decreasing pH values increases the sensitivity of microorganisms to EW because the external layer of bacteria become more susceptible to HOCl therefore their growth is inhibited (Rahman *et al*., 2016). Chlorinated water had high ORP and high pH value. These results were quite in accordance with Hung *et al*. (2010) who stated that chlorinated water has pH between 8.2 to 8.4 and the ORP value from 668.8 to 715.1 mV.

As seen on Table 4, the highest and the lowest DO value were found in AEW and tap water, respectively. The results were in accordance with Huang *et al*. (2008) and Rubik (2011). According to Hsu and Kao (2004), the amount of DO is related to chlorine bonds, especially hypochlorous acid. Hypochlorous acid was formed in AEW therefore the highest DO was found in AEW. Therefore, it can be deduced that the higher the amount of dissolved oxygen probably means the higher the antimicrobial activity of EW.

4.2. Treatment and Storage Condition

Cherry tomatoes were washed twice using orbital shaker for 5 minutes at 150 RPM. Agitation process was performed in order to improve the effectiveness of washing solutions in reducing the number of microorganisms on the surface area of fruits (Hricova *et al*., 2008). According to Hung *et al*. (2010), longer treatment time (5 min) always resulted in higher reductions in pathogen population. After being washed, cherry tomatoes were put on PET plastic trays sized 15.7cm x 13cm x 6cm. Packaging is important to maintain food quality, reduce postharvest losses, and protect food from physical, chemical, and microbiological (Opara and Mditshwa, 2013). PET has strong properties, transparent, and more resistant to high temperature and chemical compounds. PET also acts as a gas barrier which can inhibit the respiration process (Shin and Selke, 2014).

Cherry tomatoes were stored for 14 days at room temperature storage (25ºC) and 60-70% RH. Tomato is a climacteric fruit therefore it will be ripen and deteriorate quickly at room temperature storage. The normal physiological processes such as respiration, transpiration, and ethylene production are leading to deterioration (Pinheiro *et al*., 2013). Respiration process can determine the shelf life of fruit tomato. The respiration rate can be affected by several factors such as light, radiation, temperature, the presence of pathogenic microorganisms, and so forth (Bachman & Earles, 2000).

Transpiration is a process of water evaporation from tissue since the fruits were harvested. The loss of water from tissue can cause weight loss, wrinkle, colour degradation, and texture breakdown (Pinheiro *et al*., 2013). Leonardi *et al*. (2000) stated that higher storage temperature will increase the vapour pressure difference between a fruit and its surrounding air. If the pressure difference is high, it would accelerate the movement of water vapour from the fruit into the surrounding air. The storage conditions used in this study increased the respiration and transpiration rates of cherry tomatoes.

4.3. Physical Characteristics

Commonly, cherry tomato has red colour and small size (Pinheiro *et al.*, 2013). Cherry tomato that has been harvested must be acceptable by consumers. The quality of tomato is determined by its colour, texture, and flavour. As seen on Table 3, the best treatment that could maintain the physical appearance of cherry tomato was AEW, followed by 100 ppm chlorinated water. Samples treated with tap water treatment were wrinkled and shrivelled after 6 days of storage and became more severe until day 14. Likewise tap water samples, BEW samples were wrinkled and shrivelled after 7 days of storage.

Cherry tomato has high water content and susceptible to shrinkage after being harvested. Shrivelling is the signs of the moisture loss. Therefore, it could be deduced that chlorinated water (100 ppm) and AEW reduce could the moisture loss. Moisture loss was reduced by decreasing the respiration and transpiration rate of cherry tomatoes and increasing the cohesion of the cell walls (Arah *et al.*, 2015). The increment of cohesion of cell walls will inhibit the moisture loss and the fruits stay firm and fresh for 11 days. AEW could delay the ripening process and decline the respiration and transpiration rate of fruits. Huang *et al*. (2008) also stated that AEW might change the electric potential of membranes and concentration of negatively charged ions. It causes reduction of cell metabolism and firmness loss. During storage, the colour of cherry tomato became darker in all treatments. It happened because lycopene as the red colour pigment will accumulate gradually during ripening stage (Khairi *et al*. 2015).

4.3.1. Weight Loss

Cherry tomato is a perishable food because it has high water content (±94%) and still alive after being harvested (USDA, 2005). Weight loss is one of the most important physical parameters that can be used to determine fruit quality loss. Weight loss can be caused by respiration, transpiration process, and microorganisms attack. Moisture loss from fruit's tissue is caused by transpiration process due to evaporation while dry matter loss is caused by respiration process (Pinheiro *et al.*, 2013). Respiration can cause weight loss because a carbon atom was loss from the fruit to produce carbon dioxide molecule which made from an absorbed oxygen molecule and evolve into atmosphere (Gharezi *et al*., 2012).

As seen on Table 5, the percentage of weight loss increased with storage period in all treatments. On day 14, the lowest and the highest weight loss value was found in AEW samples and tap water samples, respectively. It can be concluded that AEW treatment reduces the metabolic rate of cherry tomatoes and inhibit the moisture loss during storage. Besides physiological processes, weight loss can also be caused by microorganisms attack. The best treatment was AEW which has the highest ORP value that contributed in its antimicrobial activity. The higher ORP value is usually followed by higher chlorine residues which posses antimicrobial activity (Park *et al*., 2004). Because of its antimicrobial activity, AEW could eliminate the microorganisms that can cause losses during storage (Barkai-Golan & Phillips, 1991). Tap water does not contain antimicrobial activity compare to the other washing solutions used in this study. Thus, the weight loss percentages of tap water samples were the highest of all treatments during storage.

4.3.2. Colour Difference (ΔE)

Colour is one of the most important external characteristics to determine the level of consumer acceptance. Delta E value was used as an indicator to see the differences between two objects. During storage, the values of ΔE varied from 4 to 8 and there was no significant difference between treatments. The results were in accordance with Bari *et al*, (2013) who stated that there is no significant influence of tomato's colour after being treated with different washing solutions. A delta E value between 3 and 6 usually considered as an acceptable number which means that there is an obvious differences with control. When the value of ΔE more than 6 it means that there is a very obvious difference with control (Cserhalmi *et al*., 2005).

A good cherry tomato has red colour, not only shows the maturity stage but also the desired level of flavour (Pinheiro *et al*., 2013). Red colour of cherry tomato appeared because the presence of carotenoid compounds such as ß-carotene (7%) and lycopene

(87%). The colour of cherry tomato became darker along with storage period. It was also supported by decreasing the value of Lightness (L*) in the samples during storage (see appendix 1). These results were in accordance with Khairi *et al*. (2015) who stated that tomato in light red stage of maturity (ripening stage) will significantly change during storage. Tadesse *et al*. (2015) also stated that higher temperature storage will induce a fast change in the fruit's colour.

4.3.3. Texture

Texture (hardness) is one of the most important factors which used as an indication of fruit quality. Hardness was measured because it indicates the strength of tomato fruit skin and pericarps toughness (Kinzey and Norconk, 1990). Changes of cherry tomato texture during storage can be influenced by flesh hardness and skin strength. The fruit texture becomes softer during ripening process and increase the rate of decay (Pinheiro *et al*., 2013). AEW is the best treatment that can maintain the texture of tomato up to 10 days of storage, based on Agrofarm's standard, and then followed by 100 ppm chlorinated water samples. Samples treated with tap water were loss the texture on day 7 of storage. As seen on table 7, the hardness value of cherry tomato decreased with increasing storage period in all treatments. According to Alia-Tejacal *et al*. (2007), tomato tissues softening is caused by weight loss, turgor loss, and/or enzymatic activity such as *pectin methylesterase* (PME, E.C.3.2.1.11) and *polygalacturonase* (PG, E.C. 3.2.1.15).

Based on the results, it can be deduced that AEW could extend the shelf life of cherry tomatoes at ambient temperature storage. AEW treatment could delay the ripening process and decline the metabolic rate of fruits therefore the weight loss will be minimized. AEW could inhibit the reduction of fruit's hardness loss because it might change the electric potential of membranes and concentration of negatively charged ions on fruit's cell wall. The increment of cohesion of cell walls can inhibit the moisture loss (Huang *et al*., 2008). Thus, the fruits stay firm and fresh up to 10 days. Samples treated with 100 ppm chlorinated water also stay firm and fresh up to 10 days. It happens because chlorine contains calcium that can enhance the cell wall integrity, protect cells from enzymes degradation, and increase cell wall cohesion.

4.4. Chemical Characteristics

4.4.1. Water Content

The most important constituent of cherry tomato is water. As shown on Table 9, the water content values of cherry tomato on day 0 were around 94% in all treatments. These results were in accordance with USDA (2005). As increasing the storage period, the water content of samples decreased in all treatments. Water loss can severely degrade the quality of fruit which means larger weight loss and reduce profit (Bachmann, 2000). Water loss not only affects direct quantitative loss but also loss in visual appearance (shrivelling and wilting), textural, and nutritional quality (Kader, 2002).

Transpiration process is the main cause of water losses in tomato. Storage condition like temperature and RH affect the transpiration rate of fruit. Storage with low RH can result in shrivelling and may be the evidence of water loss in fruit tissue (Arah *et al*., 2015). Leonardi *et al*. (2000) stated that higher storage temperature will increase the vapour pressure difference between fruit and surrounding air. If the pressure difference is high, it would accelerate the movement of water vapour from the fruit into surrounding air.

On day 14 of storage, the highest water content was found in AEW samples. It can be deduced that AEW can decrease the rate of transpiration process therefore the water content was still high at the end of storage time compared to the other treatments. According to Parsons *et al*. (2013), naturally, cherry tomato has an outer membrane that can minimize water loss, it is called cuticle. Cuticle is more soluble in lipid than in water. Therefore, washing process should not affect the cuticle membrane of cherry tomato. Cuticle can reduce shrivelling and extend the shelf life of fruit and improve visual fruit appearance (glossy fruits).

4.4.2. Titratable Acidity

The maturity of fruits can be determined by its acidity. The changes in organic acids during ripening were caused by increasing citrate and decreasing malate acids (Gharezi *et al*., 2012). The highest amounts of acid content in tomato are found in the form of citric acid and malic acid (Anyasi *et al*., 2016). Titratable acidity is the total or potential acidity which indicates all aggregate acids and the sum of all volatile and fixed acids. As seen on table 10, the percentages of titratable acidity decreased gradually in all treatments. Based on the results, treatments did not affect the titratable acidity percentages of cherry tomato during storage.

During storage, fruits might utilize oganic acids as the substrate of respiration process therefore the acids content in fruits decreases. Moreover, the faster respiration rate will accelerate the decline in acid levels (Tadesse *et al*., 2015). The storage condition of this study was high temperature and low RH. Both conditions will increase the respiration rate of cherry tomatoes. On day 14, the highest titratable acidity was found in AEW and 100 ppm chlorinated water samples while the lowest was found in BEW samples, followed by tap water samples. However, there was no significant difference between treatments therefore different washing solution did not affect the titratable acidity of cherry tomatoes (Gharezi *et al*., 2012).

4.4.3. Total Soluble Solid (TSS)

TSS analysis was done by using a digital refractometer. The principle of refractometer is light refraction, in which when the light comes through solution, its speed will decrease. This principle is used to determine the amount of solutes in a solution. The index of refracted solid is also known as %brix or ºbrix. Functionally, the index is based on the changes in the direction of light while passing through the solution. In general, the greater the amount of solid in the solution, the more light is bent as it passes through the solution. All dissolved compounds affect the refractive index of the solution. The higher the value of refractive index, the higher the amount of soluble solids in solution (Harril, 1998). Soluble solids levels influence the commercial use and consumer's reaction to many fresh fruit and vegetables (Matthew and Bumgarner, 2012).

Sugars contained in tomato are mostly fructose and glucose where the amount of fructose in slightly higher than glucose. Total soluble solid is a rough index of sugar content in fruits. According to Beckles (2012), TSS consists of sugars (80-85%), acids, soluble minerals, and the other minor components such as phenol, amino acids, soluble pectin, and so forth in the tomato fruit pulp. These results showed that TSS values increased continuously during storage with a range from 5 to 6.9%brix. The sugar content in samples increased because of the metabolism of organic acid into carbon-dioxide and degradation of polysaccharides to water soluble sugars and other components that contribute to soluble solids (Anyasi *et al*., 2016).

Cherry tomatoes washed in AEW had the lowest TSS value while the highest TSS value was found in tap water samples. However, there was no significant difference between treatments therefore different washing solution did not affect the TSS value of cherry tomatoes. According to Getinet *et al*. (2011), there are several factors that affect the sugar level in tomato such as variety, maturity stage, and environmental condition (Getinet *et al*., 2011). Based on Table 12, it was known that titratable acidity and total soluble solids had strong negative correlation coefficient which means the higher the titratable acidity value, the lower the total soluble solids and vice versa.

4.5.Microbial Characteristics

Microbial analysis was done by using the Total Plate Count (TPC) method and direct plating of cherry tomato's calyx. Before the microorganisms are grown in media, the sample must be diluted. Dilution technique was used in order to reduce the number of microbes in the samples. After incubation process, the numbers of microorganism colonies were calculated. Based on the results, tap water samples were the highest number of colonies in both media, followed by BEW samples. AEW and 100 ppm chlorinated water samples did not show any changes even though both of them were already used for washing. It can be deduced that 100 ppm chlorinated water and AEW had strong antimicrobial activity, therefore the microorganisms dead and it could not be detected. The obtained results seem similar with the study conducted by Fishburn *et al*. (2012) who found that AEW and chlorine were effective for reducing pathogens in tomatoes.

According to Huang *et al*. (2008), bacteria usually grow well in pH ranging from 4 to 9. Aerobic bacteria usually grow at ORP on a range from +200 to 800 mV while anaerobic bacteria grow mostly at -700 to +200 mV. Therefore, BEW and tap water have suitable conditions for the growth of bacteria. AEW had the lowest pH which not suitable for the growth of most bacteria. Based on the results, the highest colonies of microorganisms were found in tap water and BEW samples. It happened because BEW was the lowest ORP value and the highest pH value and tap water only removes dirt like dust and soil but can not eliminate microorganisms (Brackett, 1992 in Koseki *et al*., 2004).

Direct plating testing was done by isolate the calyx of cherry tomato in PDA media. PDA media was used because tomato fruit is more susceptible to fungi than bacteria (Barth *et al*., 2009). Calyx was chosen because it is the main source of potential spoilage microorganisms in tomato. Khalegi *et al*. (2013) stated that the highest bacterial and fungal counts were detected on the calyx of tomato and fungi usually appear visually on it. Moreover, the main defect of cherry tomato is mouldy stem and/or decayed stem (USDA, 2005). Based on the results, the probable spoilage microorganisms that found in the direct plating test were *Aspergillus sp*, *Penicillium sp*, and *Saccharomyces sp*.

Growth of fungal/yeast colonies was different among treatments. Based on table 14 and Appendix 2, the best treatment to control the fungal/yeast growth was found in AEW, followed by 100 ppm chlorinated water samples. BEW treatment was slightly more effective than tap water samples. AEW treatment could control the fungal growth up to 12 days of storage. Antimicrobial activity of AEW was strong because of its ORP and pH. AEW contains high ORP so oxidation could occur, harming various layers of cells and disturbing metabolic pathways inside the cell which can inactivate bacterial cells (Liao *et al*., 2007).

In this study, the pH of AEW was 3.19 therefore it could not be categorized as strong AEW but still sufficient to inactivate the microorganisms. It is also supported by Rahman *et al*. (2012) and Park *et al*. (2004) who stated that a very low pH may not be necessary for bacterial inhibition during EW application. Besides high ORP and low pH, AEW also had high DO which also indicates higher antimicrobial activity. An additional treatment like agitation can increase the bactericidal efficacy of EW, especially AEW to inactivate microorganisms. According to Park *et al*. (2004), it is occurred because chlorine residues in EW can be spread throughout the fruit surfaces and remove the microorganism cells that were attached to the surface area. They become less resistant therefore the inactivation will become easier.

This study used 100 ppm chlorinated water at pH 8.73 with 883.3 mV of ORP. Based on the results (see Appendix 2), the fungal growth colonies in 100 ppm chlorinated water samples increased after 5 days of storage. Therefore, it can be deduced that AEW was more effective than 100 ppm chlorinated water in controlling the growth of fungi in cherry tomatoes. According Suslow (2000), chlorinated water posses antimicrobial activity because of the free chlorine residues are high. However, the efficacy of chlorine is also influenced by its pH. The pH value of chlorine used in this study was high. When the pH value higher than 8.0, the disinfection process will be reduced. The lower effectiveness occurs because the level of HOCl drops to less than 20%. HOCl is the antimicrobial agent in chlorine and it will act faster at pH 6.5 to 7.0. Moreover, chlorine can produce mildly toxic chloramines when reacts with organic nitrogen-based material at high pH. Hricova *et al*. (2008) also stated that chlorine compounds which react with proteins will form organo-chloramines which weaken the antimicrobial activity.