The influence of ozone exposure on organoleptic and chlorophyll levels of curly lettuce (Lactuca sativa L.)

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The influence of ozone exposure on organoleptic and chlorophyll levels of curly lettuce (*Lactuca sativa* L.)

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ABSTRACT

Ozone has been utilized widely for sterilization application, for example to keep the freshness of both fruits and veggies yet keep them safe too for human consumption and chemical-free. This work aims to investigate the effect of ozone exposure through the air and water on organoleptic and chlorophyll level of curly lettuce (*Lactuca sativa L*). The given exposure has been done with various ozone concentrations corresponding with increasing exposure time, namely 0.0147 mg/L (120 second exposure time), 0.0294 mg/L (240 second exposure time), 0.0371 mg/L (360 second exposure time) and 0.0479 mg/L (480 second exposure time). The curly lettuce (*Lactuca sativa* L) was originally taken from veggies farmland located in Sumberojo village, Batu sub-district, Batu city East Java, Indonesia. This project was carried out by using Completed Random Contrivance (CRC) technique. The observation was focused on organoleptic and mass loss of curly lettuce (*Lactuca sativa* L) and it was carried out on every 8 hours for two days straight non-stop. The chlorophyll level measurement was employed by using UV-Vis spectrophotometer after two days from the last observation. Organoleptic showed that ozone exposure technique through water mediator with ozone concentration 0.0147 mg/L gave the best results to detain organoleptic damages of curly lettuce (*Lactuca sativa* L). The chlorophyll level was analyzed by employing *Statistical Package for The Social Sciences* (SPSS) with factorial technique (Two Way ANOVA). Therefore, all the analysis result convinced that this ozone exposure technique with water mediator gave the best results compared to air mediator, in term of chlorophyll level preserving of *Lactuca sativa L*. This technique is very effective to maintain the quality and curly lettuce (*Lactuca sativa* L) freshness.

***Key words**: Ozon, Dried lettuce (Lactuca sativa L), Organoleptic, Clorophyll level*

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Introduction

Geographically, Indonesia is located between two continents, Asia and Australia, and two oceans, the Pacific Ocean and the Indian Ocean. While astronomically, Indonesia is located at 6° NL – 11° SL and 95° EL - 141° EL. Since its location Indonesia becomes one of the 14 countries crossed by the equator. Due to this astronomical location, Indonesia has a tropical climate that has plentiful rainfall and fertile soil structure. As this soil fertility, Indonesia has abundant flora wealth. One of them is vegetables (Mattos *et al*., 2014).

Curly lettuce is one type of horticulture plant with high economic value (Nübling *et al.,* 2019). This is represented from the high number of markets demands to meet market needs, especially in hospitality, large restaurants to foreign countries as export commodities (Adimiharja *et al.,* 2013). But lettuce is one type of vegetable with a low resistance, thus it cannot last long. In spite of lettuce leaves have interesting appearance, they are often used as decorations in serving food and have crispy tastes that are very suitable for fresh vegetables, yet they are easy to rot (Adimihardja *et al.,* 2013). Hence the high economic value of lettuce is not commensurate with its cell durability.

Cell damage is induced by water content that begins to decrease after being picked as well as the alleged existence of microorganisms in the form of bacteria that grow on lettuce. In addition, cell damage is also caused by enzymes, temperature, air and light (Wulfkuehler *et al.,* 2014). As a result, lettuce does not last long, thereby reducing its economic value. The economic value is the comparison of costs incurred with the costs received. If lettuce is easily damaged, the economic value will also be reduced. This will harm the lettuce farmer. Besides the reduced value of exports will reduce the value of state income.

Storing fruits and vegetables to keep them fresh have been carried out with several ways, such as gamma-ray exposure, conventional chlorine or chlorine disinfectant (Das *et al.,* 2012). However, gamma ray radiation is expensive, the scent of chlorine is irritating and cause side effects in the form of carcinogenic trihalomethane compounds (Astuti *et al.,* 2020). Compared with chlorine, ozone speeds are 3250 times faster in killing organisms and 150% stronger in oxidative power. In addition, storing fruits and vegetables can be in the refrigerator, yet the bacteria in vegetables are still active (Baur *et al.,* 2004). Chemicals are also used to storing vegetables, but the usage of chemicals produces is harmful since it contains toxins. Another way to storing fruits and vegetables is using immersion in a solution of acetic acid ($C_2H_4O_2$) or often called vinegar water. This solution is food safe, yet the taste of food will change to be acidic. Hence, currently the ozone method is being developed which are considered to be more eco-friendly and safe (Karaca and Velioglu, 2014).

Ozone is the smallest part of the Earth's atmosphere that is unstable and quickly decomposes at ambient temperatures (Take *et al.,* 2014). Ozone is naturally formed from ultraviolet (UV) radiation from sunlight (Bocci *et al.,* 2006). Various research stated that ozone can be utilized in photodynamic therapy (Astuty *et al.,* 2018; Sunarko *et al.,* 2017; Puspita *et al.,* 2020) to inactivate the beneficial bacteria in order to prevent the population of pathogenic bacteria on the skin and food. Ozone in the mechanism of photosensitization acts as oxygen which absorbs light energy to form radical oxygen species (ROS) that will kill both planktonic bacteria (Astuti *et al.,* 2017), biofilms (Astuti *et al.,* 2017; Shie *et al.,* 2019) and fungi (Astuti *et al.,* 2019). According to Asgar *et al.,* (2017) ozone method can also shed the remnants of pesticides and heavy metals attached to the fruit or leaves that it is food safe and ozone treatment can increase the life-extending of tomatoes. Ozone can inactivate microorganisms without leaving chemical residues and will re-create into oxygen, therefore it is very eco-friendly (Syafarudin *et al.,* 2013).

The most important mechanism of ozone is the sulfhydryl oxidation from the enzyme. This layer is the first subject to be attacked by ozone. Ozone invasion of the cell wall leads to changes in the permeability of cells and causes cell lysis (Asgar *et al.,* 2017). There are several studies indicated that ozone can be used as a natural preservative. As ozone contains antibacterial which can inactivate microorganisms thus extending the storage period (Zorlugenç *et al.,* 2008). Ozone as the most powerful oxidizer after hydroxide radicals (OH *), can oxidize heavy metals, degrades organic compounds including organo-chlorides and aromatics, eliminate color, odor, taste. Justification of parameters and the best treatment for flower cabbage, namely at 1.5 mg/L ozone concentrations with leaves in cold storage due to the characteristic of ozone which acts as a sterilizer (ozone can inactivate microbes such as pathogenic bacteria, fungi, and viruses), deodorization (removes smells owing to microbes and organic compounds), decolorization (removes organic dyes) and degradation (decomposes organic compounds and oxidizes heavy metals) (Borelli nd Bocci, 2018).

Ozone exposure techniques applied in this study are exposure through air mediator (gasification) and through water mediator. Ozone exposure to red chili can increase its life-extend. Hence in this case ozone is engaged for sterilization (Calvosa *et al.*, 1991). Besides cell damage is caused by breakage to enzymes due to oxidation by ozone that initiate through the stomata, thus respiration becomes slow and water levels are not easily lost as a result of the vegetables will remain fresh (Asgar *et al.*, 2011).

Based on the introduction and existing research, this study aims to investigate the concentration and radiation techniques that are most effective in inhibiting cell damage in curly lettuce (*Lactuca sativa L*).

Materials and Methods

This study was conducted at the Analytical Chemistry Laboratory, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga for characterization of ozone concentration and Biophysical Laboratory, Department of Physics, Faculty of Science and Technology, Universitas Airlangga for ozone exposure on curly lettuce (*Lactuca sativa* L) and testing chlorophyll level of curly lettuce (*Lactuca sativa* L).

Sample Treatment

The sample used in this study was 81 curly lettuce (*Lactuca sativa L*) obtained from the Vegetable Garden in Sumberejo Village, Batu sub-district, Batu City. The study was conducted with a Completed Random Contrivance (CRC). The sample consisted of group C: control without treatment, G1 : ozone exposure through air mediator with concentration of 0.0147 mg/L (120 s exposure time), G2 : ozone exposure in the air concentration of 0.0294 mg/L (240 s exposure time), G3 : ozone exposure through air mediator with concentration of 0.0371 mg/L (360 s exposure time), and G4 ozone exposure through air mediator with concentration of 0.0479 mg/L (480 s exposure time), W1 : ozone exposure through water mediator with concentration of 0.0147 mg/L (120 s exposure time), W2 : ozone exposure through water with concentration 0.0294 mg / L (240 s exposure time), W3 : ozone exposure through water with concentration 0.0371 mg/L (360 s exposure time), and W4 : ozone exposure through water with concentration of 0.0479 mg/L (480 s exposure time).

The study was conducted with 3 repetitions. Exposure through air mediator was held by placing the sample in an isolated container and then ozone was exposed according to the time needed to reach the desired concentration. Ozone exposure through water mediator was done by exposure the ozone in 3 liters of water according to the time needed to reach the desired concentration. The sample then soaked in ozone for 6 minutes. Washing and soaking vegetables and fruit in water gives the best results between 5-10 minutes.

Organoleptic and chlorophyll observation

Organoleptic observation of curly lettuce was carried out every 4 hours for two days. Chlorophyll levels were tested after 2 days using a UV-Vis spectrophotometer (Milenkoviæ *et al.*, 2012 ; Torres *et al.*, 2016).

Statistical Analysis

Quantitative data analysis was performed using Statistical Package for Social Science (SPSS) analysis in the form of factorial tests (Two Way Anova). The data tested statistically are chlorophyll level. This test aims to determine whether there are differences in chlorophyll level in curly lettuce (*Lactuca sativa* L) after treatment.

Results and Discussion

The results of statistical testing of curly lettuce chlorophyll (*Lactuca sativa L*) levels indicate that there is an impact of exposure techniques on chlorophyll level with p value = 0.02 ($p < 0.05$), there is no effect of variations in ozone concentration on chlorophyll content with p value = 0, 65 ($p > 0.05$) and there is no influence of the interaction of the two factors on chlorophyll content with p value = 0.58 ($p > 0.05$). The results of testing the exposure technique with the Independent sample T-Test showed the technique of exposure to ozone through water mediator produced the highest average value of 2.18.

The Effect of Ozon Flow Time on Ozone Concentration

The value of ozone concentration (O_3) for each flow time is illustrated in Figure 1. Based on the graph, it

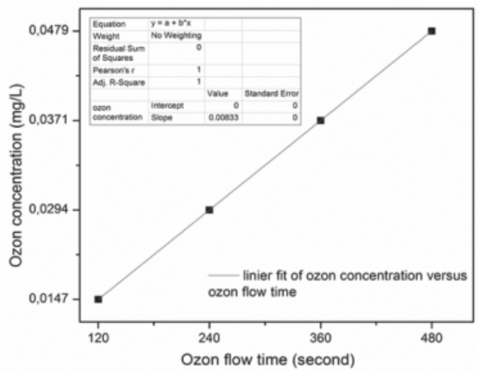

Fig. 1. The graph of ozon concentration againts ozon flow time

represents that the greater the ozone concentration time, the greater the flow.

Organoleptic

Based on observations, it stated that organoleptic curly lettuce (*Lactuca sativa* L) which was not exposed to ozone or control samples began to show appearance of wilting leaves at intervals of 16 hours and increased with time. Curly lettuce (*Lactuca sativa L*) exposed to ozone through the air mediator can be seen in Figure 2.

Observations indicated that curly lettuce (*Lactuca sativa* L) exposed to ozone through the air mediator was more easily damaged than the control group without ozone exposure. There was a brownish

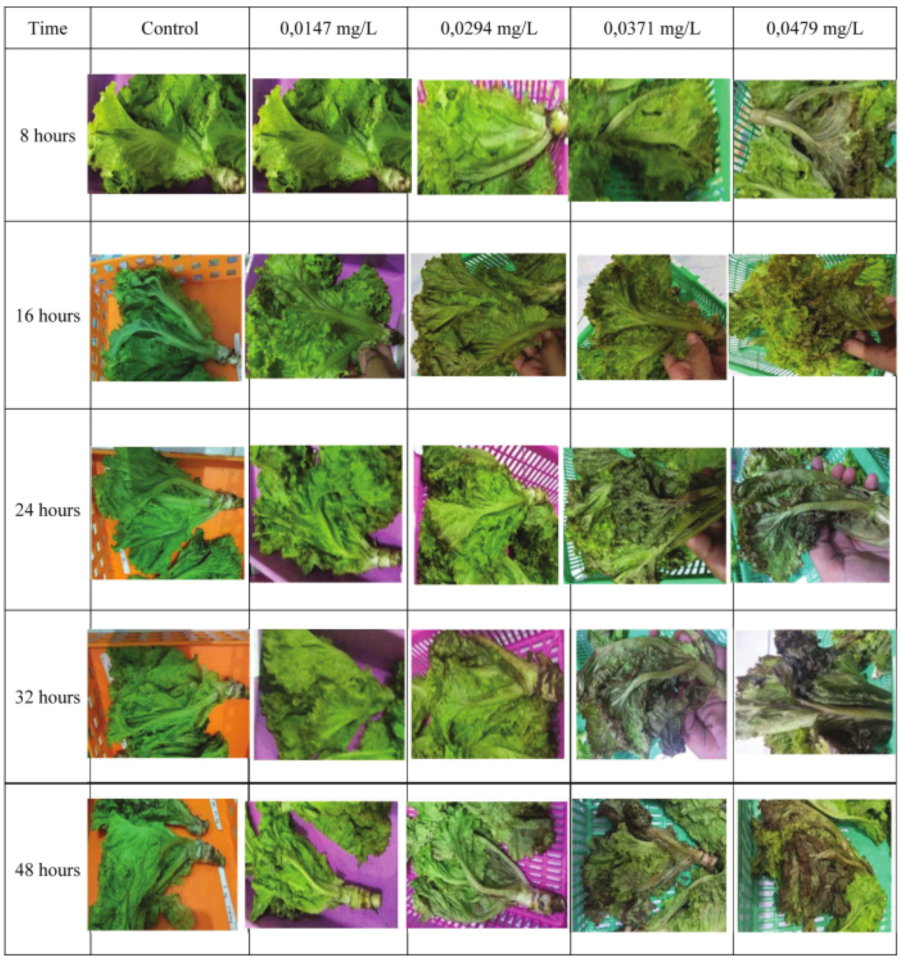

Fig. 2. The organoleptic of curly lettuce (*Lactuca sativa L*) control group with ozon exposure through air mediator

color to the leaves such as the effects of burning, wilting and drying. These effects were convenient with increasing time and concentration used. Hence the exposure to ozone through the air mediator accelerated organoleptic damage of curly lettuce (*Lactuca sativa* L). Curly lettuce (*Lactuca sativa* L) with treatment of ozone exposure through water mediator is illustrated in Figure 3.

The results of ozone exposure treatment in curly lettuce through water mediator (*Lactuca sativa* L) showed the effect of damage after 40 hours of storage in the form of leaf texture that started wilting and yellowing increased with time. Ozone exposure to curly lettuce (*Lactuca sativa* L) through water mediator are able to maintain its freshness longer than the control samples or samples that are exposed to

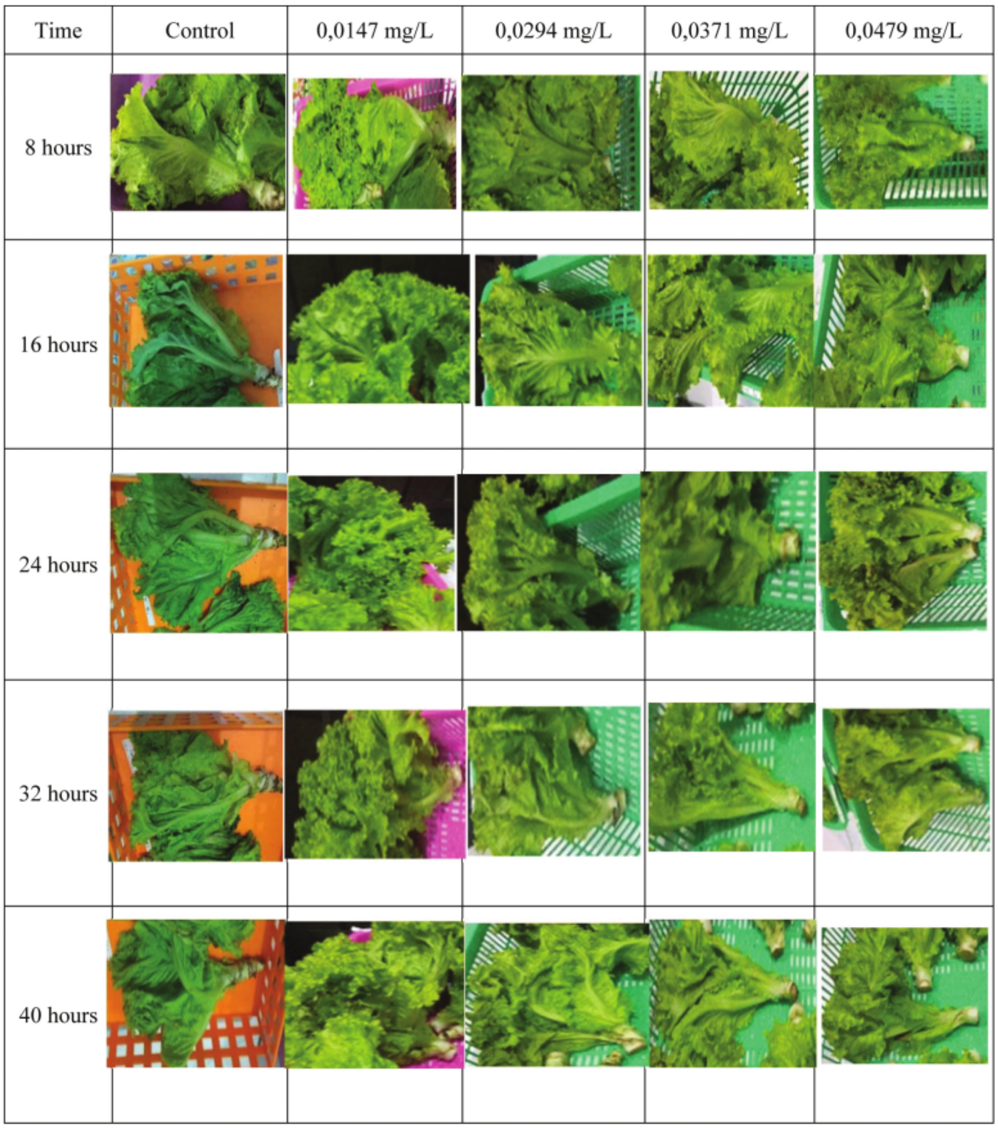

Fig. 3. The control group organoleptic and ozon exposure through water mediator to wash curly lettuce (*Lactuca sativa* L)

ozone through the air mediator. The most durable curly lettuce (*Lactuca sativa L*) was indicated by a concentration of 0.0147 mg / L.

Mass Loss of Curly Lettuce (*Lactuca sativa* L)

Graph of mass loss curly lettuce (*Lactuca sativa* L) with ozone treatment through air and water mediator is showed in Figure 4 and 5. Both graphs in Figure 4 and 5 represent that the mass loss of curly lettuce (*Lactuca sativa* L) on ozone exposure through air mediator and water mediator tends to be the same for all concentrations. Thus, in this study the weight loss cannot be applied as a reference in investigating the effective treatment. This is suspected due to the concentration used is too small hence it does not provide a significant difference.

While mass loss of curly lettuce (*Lactuca sativa* L) after ozone exposure through water mediator is illustrated in Figure 6.

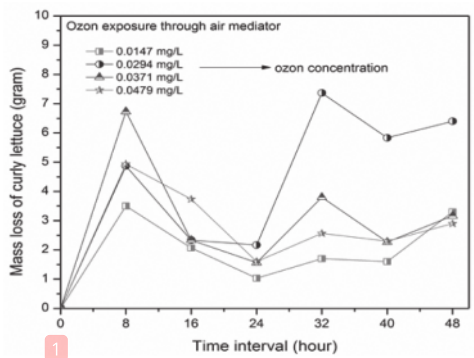

Fig. 4. Mass loss graph of curly lettuce (*Lactuca sativa L*) after ozon exposure treatment through air mediator against time interval

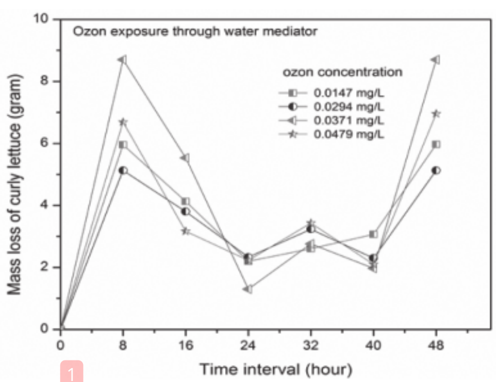

Fig. 5. Mass loss graph of curly lettuce (*Lactuca sativa L*) after ozon exposure treatment through water mediator against time interval

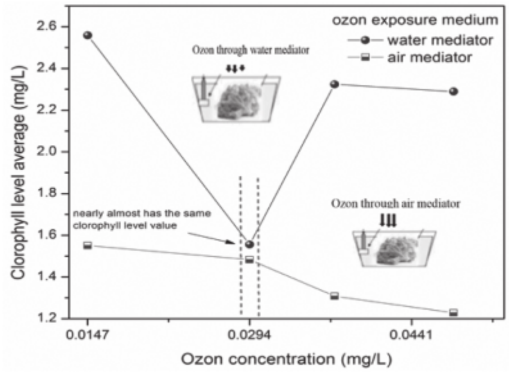

Fig. 6. The graph of chlorophylls level on ozone exposure sample for both applied mediator (water and air) versus ozone concentration

Chlorophyll Level of Curly Lettuce (*Lactuca sativa* L)

Graph of chlorophyll level of curly lettuce on both samples after ozone exposure trough air mediator and water mediator are illustrated in Figure 6.

Based on the graph, it represents that curly lettuce (*Lactuca sativa* L) exposed to ozone through water mediator gives a higher value of chlorophyll level than through air mediator. The highest levels of chlorophyll are found in curly lettuce (*Lactuca sativa* L) which is exposed to ozone through water mediator with a concentration of 0.0147 mg/L which is equal to 2.559 mg/L. While the lowest chlorophyll level is found in curly lettuce (*Lactuca sativa* L) which is exposed to ozone through the air mediator with a concentration of 0.0479 mg / L which is equal to 1,228 mg/L. This is in accordance with research by Zorlugenc *et al.*, (2008) which stated that ozone exposure through water mediator was more effective than through air mediator. The mechanism of ozone exposure to curly lettuce (*Lactuca sativa* L) is displayed in Figure 7.

The interaction of ozone when added to water is by oxidizing metal ions contained in water. This leads the electron transfer process to produce ozone radical anions. Ozone radical anions will initiate the formation of ozone decomposition chains. Ozone which reacts with carbon in water would produce hydrogen peroxide and carbonyl (Calvosa et al., 1991). According to Hong (2010) the time of washing with ozone water greatly determined the efficiency.

Ozone maintains freshness by damaging the enzymes contained in cells. Ozone will infiltrate the tissue of curly lettuce (*Lactuca sativa* L) through the stomata valves in epidermis when washing with ozone. Furthermore, ozone will oxidize cell walls into lysis or rupture. This induces the enzymes in the membrane and cell nucleus in the chloroplast to be inactive, hence the process of respiration will be inhibited (Asgar *et al.*, 2011). Respiration is a metabolic process in the form of combustion of complex compounds by oxygen (O_2) which produces simple compounds such as carbon dioxide (CO_2), water and energy for synthesis reactions by cells. The inhibition of respiration will lead the vegetable water content to be maintained thereby inhibiting cell damage. Thus, it will increase the freshness of vegetables (Okpala *et al.*, 2015).

In addition, the new compounds contained in the water will interact with microorganisms that may be carried in curly lettuce plants (*Lactuca sativa* L). In line with Nurjanah (2002), plants after being picked are still doing respiration and producing a ripening hormone, namely ethylene hormone. Microorganisms stimulate ethylene production and induce the vegetables to quickly rot.

Ozone will infiltrate microbial cells, change the permeability of microbes and lead microbes into lysis or rupture (Asgar *et al.*, 2017). Some researchers have shown that only with low ozone concentration (less than 0.5 mg/L) ozone can inactivate microorganisms in water, moreover ozone is able to sterilize water (Asgar *et al.*, 2011). The sensitivity of microorganisms with ozone is affected by several factors including microorganism strains, cell physiological state, growth rate, pH of the medium, temperature, humidity (Zorlugenc *et al.*, 2008).

Ozone treatment on curly lettuce (*Lactuca sativa L*) can maintain the freshness up to 5 days compare with controls that only last for 2 days in the form of wilted leaves and soft, runny texture. Observation of curly lettuce (*Lactuca sativa* L) was carried out in every 8 hours in term of mass loss.

Mass loss is mainly due to transpiration and infrequently by respiration (Asgar *et al.*, 2011). Shrinkage of weight indicates reduced water content. Decreased water content during storage can induce vegetables wilt and decrease their fertility. In this study the weight loss does not provide a real difference allegedly owing to the concentration used is too small. Thus it does not provide a significant difference.

Curly lettuce (*Lactuca sativa* L) gives changes to the organoleptic. Organoleptic testing was carried out based on the sensing process. In accordance with this study, it is able to be discovered that there are changes in the curly lettuce such as the texture is softer and changes in the color of the leaves. Asgar *et al.*, (2011) stated that the softening of fruits or vegetables was owing to changes that occur in cell walls and other pectin substance compounds, namely the solubility of the depolimeration of pectin sub-

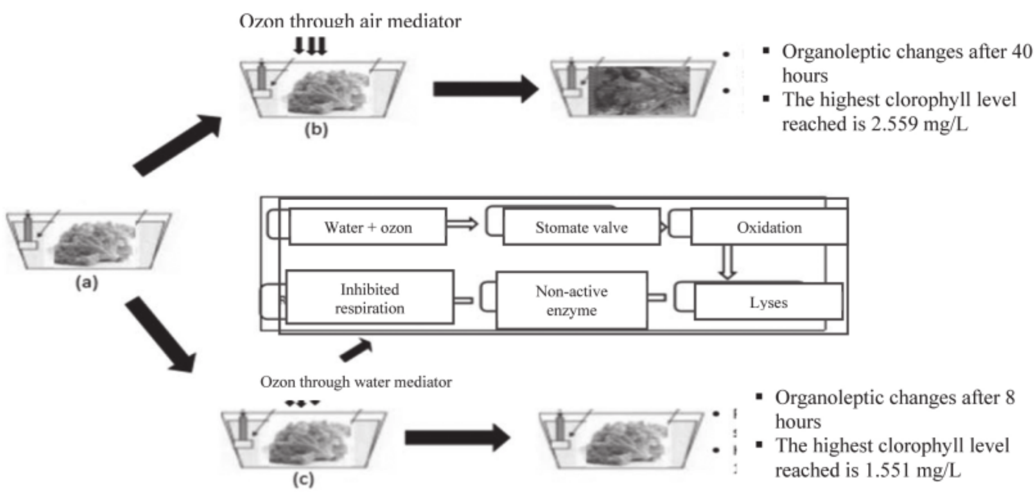

Fig. 7. Ozon exposure mechanism on curly lettuce (*Lactuca sativa* L), (a) given exposure was divided in two kind of exposure technique, (b) ozone exposure through air mediator, (c) through water mediator that inhibit cell respiration thus it can maintain its durability

stances. Insoluble pectin was transformed by various enzymes to water-soluble pectin. According to Asgar *et al.* (2011) on flower cabbage, excess oxygen from the decomposition of the remaining ozone actually became the main menu for the growth of the aloive microbes, these microbes that induced the texture of plants to be soft.

The transformation of green leaves to yellow on curly lettuce (*Lactuca sativa* L) is due to the degraded chlorophyll pigment structure which was followed by the formation or appearance of yellow pigment (Milenkoviæ *et al.*, 2012). In consonance influential factors in the formation of yellow color were abscisic and ethylene hormones. Both of these hormones triggered the aging of leaves which induced the loss of chlorophyll, RNA, protein, and stimulated the formation of carotenoids. In line with Asgar *et al.* (2011) discoloration in vegetables caused by the majority of chlorophyll pigments undergo changes during storage due to maturity, besides the loss of Mg + from the tetrapyrrole structure to mold feotin in chlorophyll induced the brown discoloration.

Chlorophyll level in curly lettuce (*Lactuca sativa* L) were tested after two days using a UV-Vis spectrophotometer. UV-Vis spectrophotometer tested the chlorophyll level by detecting the concentration of the sample solution (Gokmen *et al.*, 2002). Light would absorb the color of the sample solution at certain wavelengths. The research applied a Genesys 30 type UV-Vis spectrophotometer that could detect wavelengths in the range 325-1000 nm. The level of curly lettuce chlorophyll (*Lactuca sativa* L) was calculated using the formula Wintermans and Mots (Pratama and Ainun, 2015) which used 2 wavelengths in their analysis, namely at 665 nm and 649 nm. In this study the chlorophyll level produced from various treatments had almost the same value. This was presumably due to the difference in concentration used was too small.

Conclusion

Ozone exposure through the air mediator can accelerate organoleptic damage of curly lettuce (*Lactuca sativa L*) while ozone exposure through water mediator can inhibit organoleptic damage of curly lettuce (*Lactuca sativa* L). Nevertheless, ozone exposure through air mediator and through water mediator do not indicate an impact on the chlorophyll level of curly lettuce as it gives nearly same value. This is presumably due to the concentration range of ozone exposure was too small. The most effective treatment in preventing damage to curly lettuce (*Lactuca sativa* L) is ozone exposure through water mediator at a concentration of 0.0147 mg/L. Other parameters freshness of some specific fruits and various veggies using this technique need further investigation.

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