International Journal on Advanced Science Engineering Information Technology

Vol.12 (2022) No. 3
ISSN: 2088-5334

Bacterial Disinfection Using Ozone Gas to Extent Spinach's Shelf Life: The Effect of Dosage, Duration, and Spraying Frequency

Aprilia Ayuning Putri [a], Rais Salsa Muhammad [a], Eva Fathul Karamah [a,*]

[a] *Department of Chemical Engineering, University of Indonesia, Depok, 16424, Indonesia*
*Corresponding author: *eva.fathul@ui.ac.id*

***Abstract*— Spinach (*Amaranthus tricolor*) is one type of leafy vegetable famous as a nutrition source in Indonesia. However, it has short shelf life due to bacterial activity, which requires an alternative preservation solution besides drying. Ozone can be used as a disinfectant that is not harmful to humans and has been applied in food and agricultural product preservation. Ozone gas spraying reduces bacterial growth so that quality degradation due to decay can be slowed. In this research, spinach is preserved by spraying ozone gas to the extent of its shelf life. Spinach is ozonated with ozone gas at various dosages of 30.72 mg/hr, 48.60 mg/hr, and 80.16 mg/hr, with the spraying frequency of once, twice, and 3 times for 15 minutes. Ozone gas spraying duration is also variated: 2 minutes, 3 minutes, and 6 minutes. Then, the sample is stored for 7 days to see the change of its characteristic. The best quality during storage is shown by spinach treated with ozonation at a dosage of 48.60 mg/hr; 6 minutes; and the frequency of 3 times. In addition to variations in duration, dosage, and frequency, the same ozone exposure with different combination of duration-frequency and duration-dosage were also observed to evaluate the optimum ozone treatment for preserving spinach, and it was found that longest duration-least frequency and moderate duration-moderate dosage are preferred in maintaining the quality of spinach during storage.**

***Keywords*— Bacterial disinfection; ozone gas; preservation; shelf life; spinach.**

Manuscript received 2 Sep. 2020; revised 12 Dec. 2020; accepted 30 Jan. 2021. Date of publication 30 Jun. 2022.

I. INTRODUCTION

Leafy and green vegetables play an important role in fulfilling nutrition and maintaining health. This food class serves as one of the sources of vitamins and minerals needed by the body in supporting growth and metabolism activity [1]. Spinach is one type of leafy vegetable that is famously known as a source of nutrition in Indonesia. This leafy vegetable with the Latin name *Amaranthus tricolor* contains 39.9 g of protein, 358 mg of calcium, 2.4 mg of iron, 0.8 mg of zinc, 18 mg of vitamin A, and 62 mg of vitamin C per 100 grams of mass [2]. The average consumption of spinach is 3.81 kg/cap/year, and thus, data is projected to increase to 4.35 kg/capita/year by the year 2022 [3].

Nowadays, awareness of healthy lifestyles among the community has increased the demand for organic vegetable products, including organic spinach, even though organic vegetables cost more than the regular ones. On the other hand, although the market demand for spinach, including organic spinach, is quite high, not all regions can produce spinach to meet their market needs. Therefore, spinach producer regions distribute this commodity to meet the nation's needs. Spinach is included in the food group that is easily damaged because it has a quite high water-content which is around 86-90%. This condition is a factor that supports the growth of microorganisms, including spoilage bacteria [4].

High amounts of bacteria can degrade food quality because its growth and reproductive activity can produce by-products that will change vegetable quality. In addition, spinach is also classified as a vegetable group with a very high respiration rate [5]. The higher rate of respiration, the decomposition of starch, sugar, and organic acids take place more quickly so that the shelf life becomes shorter [6]. If not preserved, spinach has a shelf life of only less than three days. Spinach distribution activities from producers to distributors and consumers, especially for organic spinach products with better quality and higher market prices, have driven more urgency to extend the spinach shelf life. A certain preservation process is needed to maintain the quality of spinach longer. To extend the shelf life of spinach, currently, there are two often applied methods: cooling and drying. Both have weaknesses in terms of the limited ability to disinfect bacteria, limited shelf life, and potential for causing a decrease in product quality. Therefore, other alternatives are needed in preserving spinach.

Food preservation technology keeps on growing, and some developed countries have implemented ozone technology. Ozone is often implemented as a strong and safe disinfectant to control microorganisms' growth, reducing food products' shelf life [7]. The use of ozone in the food industry by direct contact is categorized as safe with GRAS (Generally Recognized as Safe) status declared by the FDA (US Food and Drug Administration) in 2001. Food industries that have applied ozone technology to reduce contamination include fresh fish products, meaty products, dairy and milk, liquid food (such as apple and orange juices), and food grain products [8,9,10,11]. Ozonation method by contacting ozone gas directly can be an alternative for preserving spinach. Contact between ozone and bacteria will kill bacteria [12]. The use of ozone in the gas phase has a longer half-life than ozone dissolved in water [13]. Besides, ozone in the gas phase will not increase the water content that can support microorganisms' growth in leafy vegetables.

In this research, preserving spinach is carried out using ozonation techniques by directly spraying ozone gas. The study involved independent variables, including ozone gas dosages, duration of ozone gas spraying, and frequency of ozone gas spraying, to evaluate the effectiveness of ozone gas direct spraying in preserving spinach. The quality parameters of spinach tested were TPC (Total Plate Count) for TMAB (Total Mesophyl-Aerobic Bacteria) content, vitamin C content, change in mass, and organoleptic feature of samples (color, odor, and texture).

II. MATERIAL AND METHOD

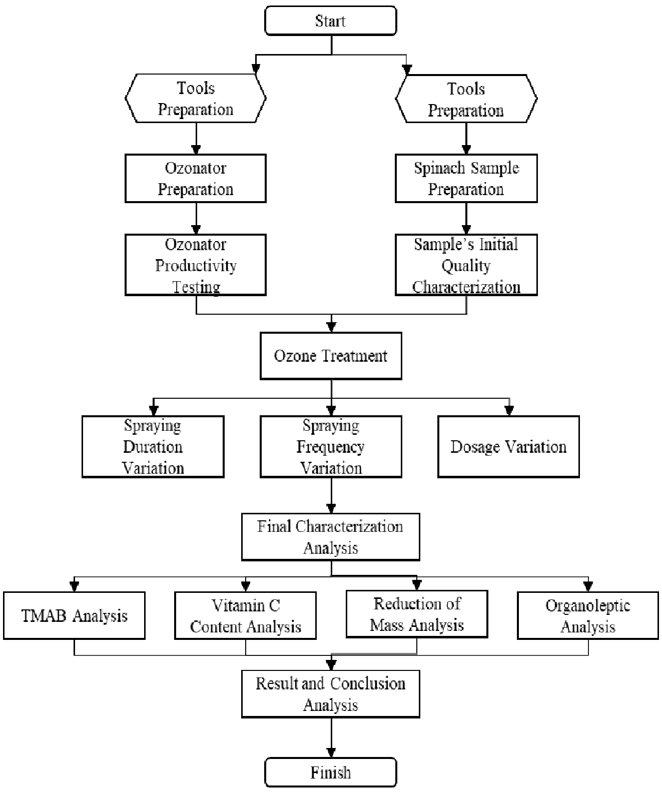

Fig. 1 Research Method Flowchart

A. Preparation

The overall research steps are shown in Figure 1. Ozonator productivity testing was carried out using the iodometric titration method. This step is done to ensure that the equipment is functioning properly, measuring the ozone productivity and setting the expected ozone production rate. All samples of spinach used in this study were taken directly from organic and hydroponic vegetable farmers known as "Sayuran Pagi" located in Beji, Depok, to represent research objectives that focus on preserving leafy vegetables from farmers to distributors or consumers. Only the leaf part of spinach with a control weight of 15 grams was used for each treatment. Before the spinach samples were treated with ozone and stored, the samples' initial quality parameters were characterized.

B. Ozone Treatment

In this ozone treatment step, variations of dosage, spraying duration, and frequency of spraying were carried out. In determining the magnitude of variation, preliminary study and trial were carried out. The dosage variations used were 30.72 mg/hour, 48.60 mg/hour, and 80.16 mg/hour, exposed with variations of spraying duration of 2 minutes, 3 minutes, and 6 minutes, with a frequency of spraying of once, twice, and 3 times for a total contact time of 15 minutes. In addition to variations in duration, dosage, and frequency, the effect of variation, including duration-frequency and duration-dosage combinations for the same estimated amount of ozone exposure, was also observed to evaluate the optimum combination of ozone treatments. The variables that were kept constant in this study were storage time, total ozonation treatment contact duration, and initial sample mass. Spinach control samples were also observed during the study as a comparison. Samples were then stored at a refrigerator temperature of 6 - 10°C for 7 days. Quality parameters of samples were characterized after ozonation and storage.

C. Final Characterization Analysis

The samples' final characterization was carried out over various time frames to observe changes in quality during 7 days of storage period.

1) TMAB Analysis: Microbiological quality of spinach was tested by counting the number of bacteria present in the spinach sample. Calculation of aerobic mesophyll bacteria number is carried out by the Total Plate Count (TPC) method. The testing principle used is according to Indonesia National Standard (SNI) 2332.3: 2015 about the determination of TPC in which microorganisms are grown by pouring method and later incubated under aerobic conditions at appropriate temperature and time until it grows and breeds by forming countable colonies. Microbiological testing was carried out at time frames of 0, 1, and 168 hours. All the results of the TPC test for spinach samples were expressed in units of CFU/gram.

2) Vitamin C Content Analysis: The type of vitamin with the largest spinach content is vitamin C. Ascorbic acid or vitamin C, is the simplest type of vitamin compared to the other types of vitamins. As a strong reducing agent, it is easily oxidized through a series of reaction mechanisms producing other substances that do not have vitamin C activity [14]. Decomposition of this vitamin can be used as an early indication for the possibility of decomposition of other more stable nutritional content such as vitamin B, vitamin A, and minerals. Analysis for vitamin C content was carried out by the oxidation-reduction titration method using iodine. Vitamin C will be oxidized by iodine forming dehydroascorbic acid while iodine is reduced as iodide ions. After all iodine reacts with ascorbic acid, the remaining iodine reacts with the starch indicator to form a black-blue color that indicates the end of the titration. Analysis of vitamin C content was carried out at 0, 24, 48, 96, and 168 hours of time frames. All vitamin C content analysis results were expressed in mass percentage unit (%) and C/Co (ratio of final vitamin C content to initial one).

3) Reduction of Mass Analysis: Analysis of mass reduction parameters is done to determine the mass changes that occur after the preservation process is carried out. The change in mass is observed by weighing the sample at 0, 1, 48, 96, and 168 hours of time frames. All the mass reduction analysis results are expressed as a percentage of the mass reduction compared to the initial mass (%).

4) Organoleptic Analysis: Organoleptic or sensory analysis consists of several sensory tests of odor, color, and texture. The analysis was done by score test method as stated in SNI 01-2346-2006. Evaluation of the samples is done by giving a certain score on the assessment sheet under the description of the product quality based on the panelist's observation result. The number of the non-standard panelist (uncertified respondents for organoleptic testing) required according to SNI is 30 people, and the number of panelists involved in this study is 38 people. Organoleptic testing was performed at initial conditions, 1, 48, 96, and 168 hours of time frames.

III. Result and Discussion

A. The Effect of Ozone Dosage

Spinach is exposed with ozone gas with a dosage variation of 30.72 mg/hour; 48.60 mg/hour; and 80.16 mg/hour for 3 minutes, once spraying frequency, ozonation temperature of 28°C and storage temperature of 8°C.

1) The Change of TMAB: The initial sample's TMAB count before ozonation treatment was 7.30 × 10^6 CFU/gram. From Figure 2, it can be seen ozone gas spraying at all variations of dosage can reduce the TPC of mesophyll aerobic bacteria. Ozone in the gas phase can disinfect bacteria by providing oxidative pressure due to the presence of oxygen-reactive species such as ·OH, ·OOH, H_2O_2 derived from ozone decomposition [1,13]. This oxidative species should normally be neutralized by the enzyme catalase produced by bacteria to protect its cells from oxidative damage [15]. However, high oxidative pressure can decrease the balance between the catalase enzyme and reactive species. This results in the accumulation of oxygen-reactive species [16], which then deactivates bacterial cells through progressive oxidation of important cell components such as nucleoids (DNA), ribosomes, cell membranes, and peptidoglycans in cell walls [13].

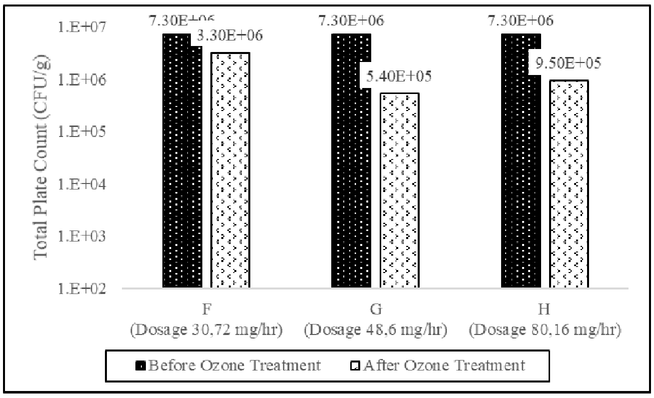

Fig. 2 The Effect of Dosage towards TMAB

Ozone gas spraying with a dosage variation of 30.72 mg/hour; 48.6 mg/hour; and 80.16 mg/hour can reduce TMAB TPC to 3.30 × 10^6 CFU/gram; 5.40 × 10^5 CFU/gram; and 9.50 × 10^5 CFU/gram consecutively. There is no linear trend between ozone gas dosage and TMAB reduction in spinach observed in this study. The biggest reduction of TMAB occurred at ozone gas dosage of 48.6 mg/hour, and when the dosage was increased to 80.16 mg/hour, there was a reduction in the quantity of bacterial disinfection, but it was still better compared to the dosage of 30.72 mg/hour. Ozonation treatment on leafy vegetables, when reaching a certain amount of ozone concentration (critical concentration), an increase in ozone concentration will not give a significant difference in disinfection ability [17]. This is probably due to the softer texture of leafy vegetable products, which limits the effectiveness of increasing ozone concentrations in deactivating bacteria [18].

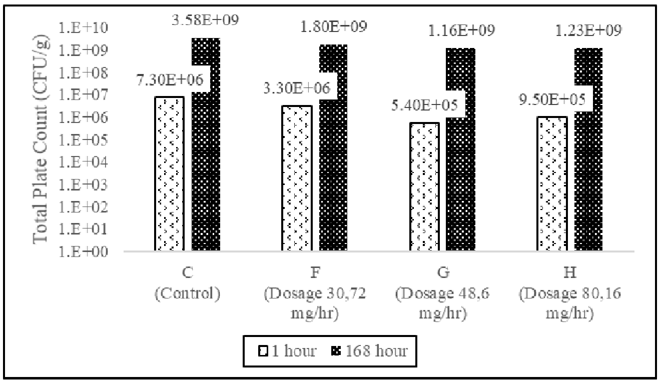

Fig. 3 The Effect of Dosage towards TMAB during Storage Time

Based on Figure 3, it can be seen the changes of TMAB content still happened even though it has received ozonation treatment. Around 10% of the pathogen population is resistant to ozonation treatment [17]. One factor that affects the difference in resistance of a bacterial cell to ozone is cell age. In addition, some types of bacteria, such as Listeria monocytogenes, are still resistant to low temperature storage [19]. All samples that received ozonation treatment had lower TMAB TPC compared to control sample both at the first hour right after the ozonation treatment and after storage for 7 days. After 7 days of storage, the highest bacterial growth was experienced by control sample that did not undergo ozone treatment at all with 3.58 × 10^9 CFU/gram, then followed by samples with ozonation treatment dosage of 30.72 mg/hour; 80.16 mg/hour; and 48.60 mg/hour with each TMAB TPC of 1.80×10^9 CFU/gram; 1.23×10^9 CFU/gram; and 1.16×10^9 CFU/gram consecutively. This is due to the condition at the first hour right after the ozonation treatment. The order of TMAB TPC of samples are the same from highest to lowest.

2) The Change of Vitamin C Content: As can be seen in Figure 4, as storage time passes, the vitamin C content in each sample decreases. During cold storage, the ascorbic acid content in vegetable decreases. This is likely due to the conversion of ascorbic acid to dehydroascorbic acid with the ascorbic oxidase enzyme's assistance [14]. Based on the data obtained after the first 24 hours of storage time, there is no significant difference in vitamin C content between control and samples treated with ozone gas with various dosage variations. This is possible because the use of ozone in food will quickly decompose into oxygen without leaving any residues or chemicals that can cause physiological changes in food [21].

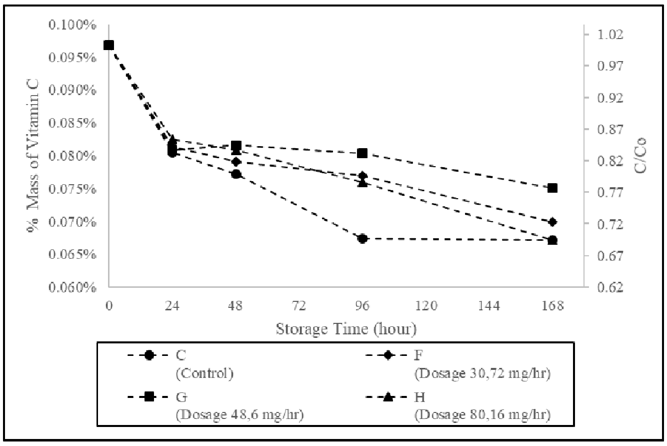

Fig. 4 The Effect of Dosage towards Vitamin C Content during Storage Time

During the storage period of 7 days, there are differences in the rate of change in the content of vitamin C between control and ozone-treated samples. It was observed from the ability to reduce the rate of vitamin C decomposition, the order from the best dosage is sample G (48.60 mg/hour); sample F (30.72 mg/hour); and H samples (80.16 mg/hour). If this data of vitamin C content is related to TMAB TPC, sample G that receives ozone treatment with a dosage of 48.6 mg/hour is also the sample with the least number of bacteria during 168 hours of storage period. The smaller total number of mesophyll aerobic bacteria is one factor that slows the rate of vitamin C reduction. Same as humans, microbes such as bacteria also need vitamins to help organisms continue to live by activating proteins needed to perform various activities [22].

3) The Change of Mass: All samples exposed to ozone gas experienced a greater mass loss compared to control as can be evaluated in Figure 5. This is because changes in the concentration of ozone gas in the container will reduce air humidity, water on the sample's surface will tend to evaporate into the air (changing from the liquid to gas phase) until the humidity balance condition achieve.

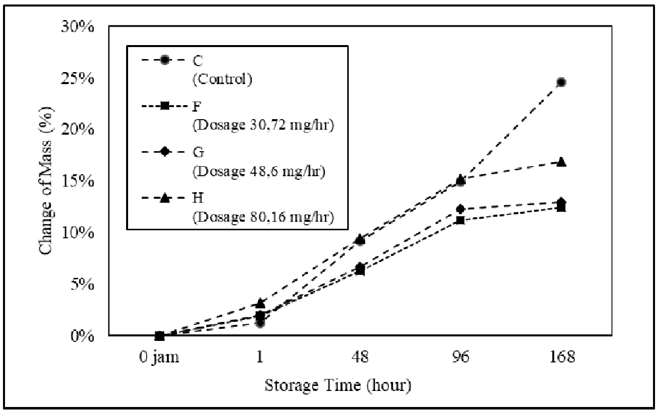

Fig. 5 The Effect of Dosage towards Change of Mass during Storage Time

During storage time, there is an observable decrease in sample's mass which indicates that after harvest, the plants continue the respiration process so that the water content tends to decrease and the product experiences withering. Respiration rate is an important indicator in determining the amount of mass loss during the food storage process. In general, the higher the intensity of respiration, the faster respiration occurs, the faster the loss of mass, including a nutrient content loss in food [6]. Besides, the change in sample's mass during storage can also be caused by the activity of decomposing bacteria in decomposing chemical compounds.

All samples treated with ozone experienced a slower rate of mass reduction compared to the control sample because they experience wilting due to slower respiration, so that the loss of water content during the storage period also takes place slower. Ozone can reduce pore size on the fruit and vegetable's surface [23], which prevents bacterial infections and water transpiration, thereby slowing down mass loss. The order of mass reduction rate from the fastest is the control sample, sample with a dosage of 80.16 mg/hour: sample with a dosage of 48.6 mg/hour, and sample with a dosage of 30.72 mg/hour. Ozone gas spraying on low and medium concentrations for spinach is better in controlling the rate of mass reduction.

4) The Change of Organoleptic Feature: Right after ozone treatment, the panelists observed white patches color changes at the sample with a dosage of 48.60 mg/hr and 80.16 mg/hr. This is due to high concentrations of ozone, which can react with color pigments such as carotenoids and chlorophyll to lead to minor discoloration right after ozonation treatment [24]. The higher the dosage of ozone used, the greater the side effects of discoloration will be. A sample with a 37.20 mg/hour dosage gives the slowest rate of color change to decay during storage time, and its quality is still above the standard while the control sample's quality has been below the standard since the 4^{th} day. Samples treated with ozone at a 48.60 mg/hour dosage experienced the slowest change in odor and texture towards decay, and its quality is above the standard until the 7^{th} day. Meanwhile, the control had begun to smell awful and change into more soft-textured.

B. The Effect of Spraying Duration

Spinach is exposed to ozone with a variation of spraying duration of 2 minutes, 3 minutes, and 6 minutes at a dosage of 30.72 mg/hour, with once spraying frequency, ozonation temperature of 28°C, and storage temperature of 8°C. The effect of spraying duration on the quality of spinach is shown in Figure 6 – Figure 9.

1) The Change of TMAB: Ozone gas spraying with a duration of 2, 3, and 6 minutes can reduce TMAB to 3.30×10^6 CFU/gram; 3.30×10^6 CFU/gram; and 8.50×10^4 CFU/gram. The trend of TMAB reduction shows that the longer the duration of ozone gas spraying, the higher number of TMAB that can be disinfected. This trend is in line with the results of [25], which concluded that an increase in the duration of ozone gas spraying will increase bacterial death due to the greater total ozone exposure.

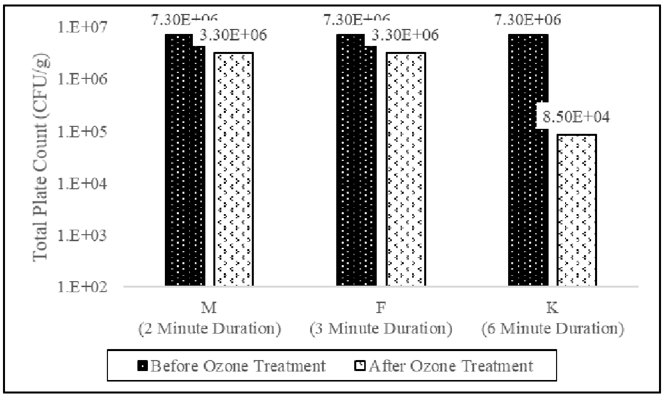

Fig. 6 The Effect of Spraying Duration towards TMAB

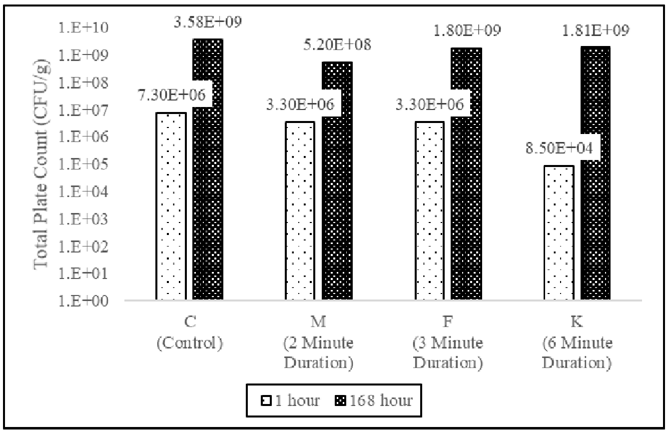

Fig. 7 The Effect of Spraying Duration towards TMAB during Storage Time

Meanwhile, inversely proportional to the first one hour data taken a right after ozone treatment, on the 7th day of storage, the longer duration of ozone gas spraying, the more TMAB detected. The sequence of bacterial counts at the beginning and end of storage should be similar. The results obtained can occur due to other factors that affect the growth and propagation of bacteria such as differences in nutrient concentrations, ion and salt content, oxygen availability, temperature, accidental contamination, and water content [26].

2) The Change of Vitamin C Content: Observing from the ability to slow down the decomposition rate of vitamin C during 168 hours of storage, the sequence of spraying duration from the best is sample K (6 minutes spraying duration), sample F (3 minutes spraying duration), sample M (2 minutes spraying duration), and the worst is control sample. It can be concluded that the longer the period of ozone gas spraying, the decomposition rate of vitamin C will be slower.

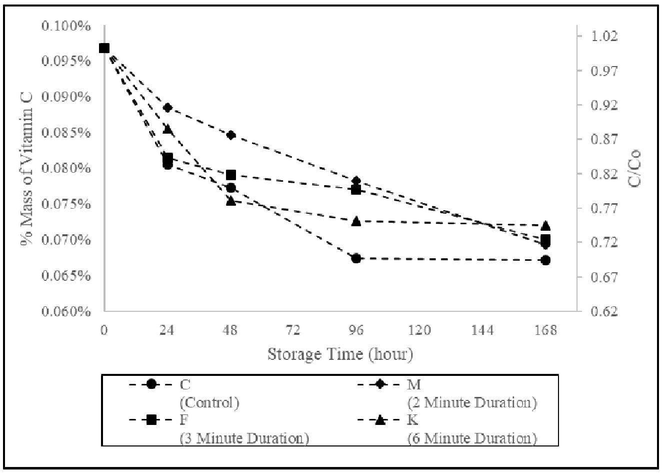

Fig. 8 The Effect of Spraying Duration towards Vitamin C Content during Storage Time

3) The Change of Mass: All samples exposed to ozone gas at various duration variations experienced greater mass changes compared to control, but the difference was not too significant.

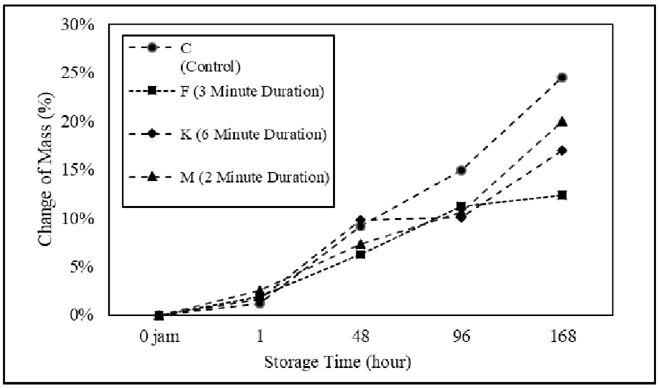

Fig. 9 The Effect of Spraying Duration towards Changes of Mass during Storage Time

The sequence of mass reduction from the fastest is the control sample, sample with spraying duration of 2 minutes, duration of 6 minutes, and duration of 3 minutes. There is no linear correlation between the duration of ozone gas spraying and the rate of mass reduction during storage time. This can be caused by the simultaneous reaction between the reactions that result in mass gain and mass loss so that the change in mass that occurs cannot be seen only as a result of water transpiration and nutrient decomposition, which decreases the quality of spinach. On the other hand, bacterial activity can also increase water content; bacteria produce water vapor as a metabolic product so that the amount of water in the sample may increase [27].

4) The Change of Organoleptic Feature: During the seven-day storage period, samples that received ozonation treatment with a duration of 6 minutes always obtained a test score of colors, odors, and textures that met the standards, and it also gained the highest score compared to the ozone-treated sample with spraying duration of 2 minutes, 3 minutes, and control sample. Control samples have shown signs of decay in terms of color since the 4th day and odors and textures on the 7th day.

C. The Effect of Spraying Frequency

Spinach is exposed to ozone gas with varying spraying frequency of once, twice, and 3 times at a dosage of 30.72 mg/hour, for 3 minutes, with ozonation temperature of 28°C and storage temperature of 8°C. The effect of spraying duration on the quality of spinach are shown in Figure 10 – Figure 13.

1) The Change of TMAB: Ozone gas spraying with a spraying frequency of once, twice, and 3 times during the total contract duration of 15 minutes can reduce the TPC of mesophyll aerobic bacteria to 3.30×10^6 CFU/gram; 1.08×10^6 CFU/gram; and 2.24×10^5 CFU/gram consecutively. This trend of reducing TPC of mesophyll aerobic bacteria shows that the more the frequency of ozone gas spraying being applied, the higher the amount of TMAB that can be disinfected.

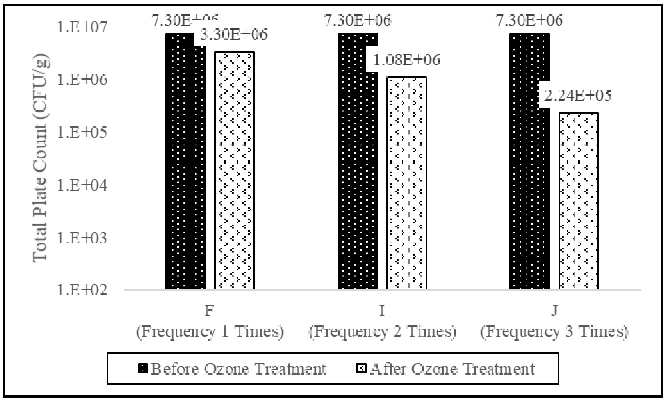

Fig. 10 The Effect of Spraying Frequency towards TMAB

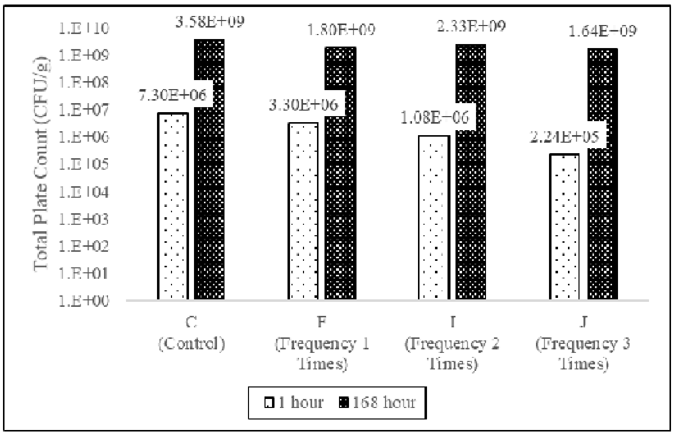

Fig. 11 The Effect of Spraying Frequency towatds TMAB during Storage Time

The best sample judged from its ability to deactivate mesophyll aerobic bacteria both in the first one hour and 168th hour of storage is a sample that are treated by ozone with a spraying frequency of 3 times. This is because in ozone gas spraying with frequency as much as 3 times the possibility that cells are physically protected by other cells on the surface during ozonation treatment. It can be avoided because in these 3 frequencies. It is possible to change the position of bacteria that increases the likelihood of contact with ozone gas.

2) The Change of Vitamin C Content: It was observed from the ability to reduce the rate of vitamin C decomposition during 168 hours of storage, the order of the spraying frequency from the best are sample I (spraying frequency twice), sample J (spraying frequency 3 times), sample F (spraying frequency once), and the worst is control sample. Based on the rate of reduction of vitamin C content during 168 hours of storage, it can be seen that the frequency of ozone gas spraying more than once can slow down the rate of vitamin C decomposition better than the once spraying frequency because the amount of total ozone gas exposure is higher.

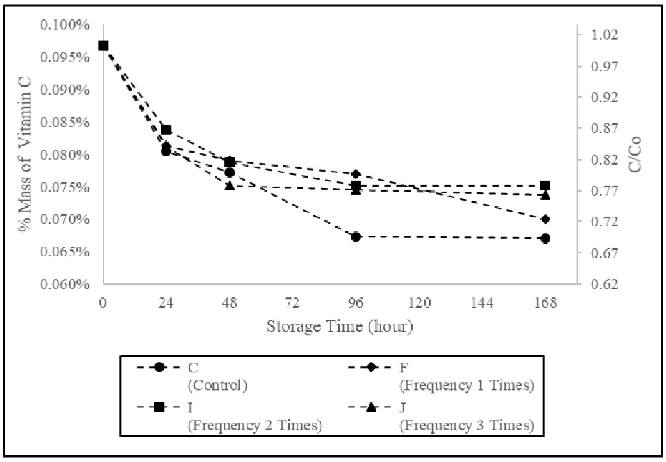

Fig. 12 The Effect of Spraying Frequency towards Vitamin C Content during Storage Time

3) The Change of Mass: All samples exposed to ozone gas at various spraying frequencies experienced greater mass changes than control samples but were not significant.

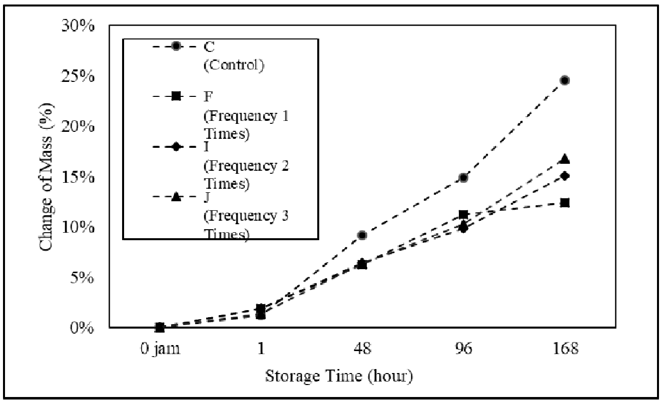

Fig. 13 The Effect of Spraying Frequency towards Changes of Mass during Storage Time

The fastest sequence of mass loss rates are control sample, frequency 3 times, twice, and once. When these results are compared with TMAB testing results, organoleptic feature observation, and vitamin C content, this trend tends to oppose. This can be caused by the simultaneous reaction between the reactions that result in mass gain and mass loss so that the change in mass that occurs cannot be seen only as a result of water transpiration and nutrient decomposition which decreases the quality of spinach. On the other hand, bacterial activity can also increase water content, bacteria produce water vapor as a metabolic product so that the amount of water in the sample may increase [27].

4) The Change of Organoleptic Feature: During 7 days of storage period, samples that received ozonation treatment with spraying frequency of 3 times and twice obtained higher color, odor, and texture scores compared to control sample and once spraying frequency sample and still met the standard. Meanwhile, on the 4th day the control sample had undergone a color change to decay and produced an unpleasant odor so that it no longer met the standards.

D. The Effect of Duration-Frequency Combination

The combination of duration and frequency varied in this study are 2 minutes - 3 times, 3 minutes - twice, and 6 minutes - once, which are based on estimated equivalent amount of ozone exposure. Fixed variables include dosage of 30.72 mg/hour, ozonation temperature of 28°C and storage temperature of 8°C.

1) The Change of TMAB: Ozone gas spraying with a combination of duration - frequency with a variation of 2 minutes - 3 times, 3 minutes - twice, and 6 minutes – once, can reduce the TPC of mesophyll aerobic bacteria to 4.30×10^5 CFU/gram; 1.08×10^6 CFU/gram; and 8.50×10^4 CFU/gram as shown in Figure 14.

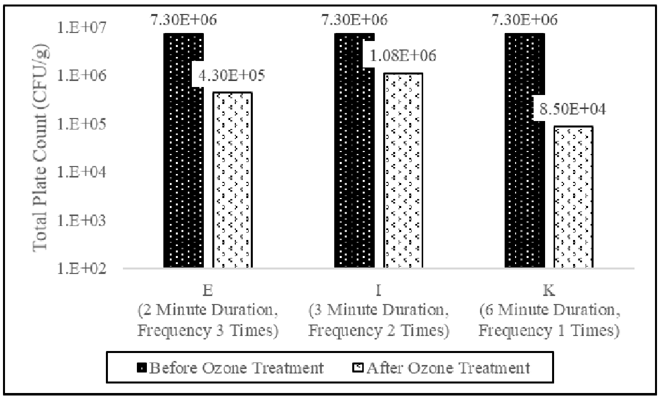

Fig. 14 The Effect of Duration-Frequency Combination towards TMAB

Ozone gas spraying with a combination of duration - frequency with a variation of 2 minutes - 3 times, 3 minutes - twice, and 6 minutes – once, can reduce the TPC of mesophyll aerobic bacteria to 4.30×10^5 CFU/gram; 1.08×10^6 CFU/gram; and 8.50×10^4 CFU/gram.

Figure 15 shows that on the 7th day of storage, there was a change in the sequence of the TPC of mesophyll aerobic bacteria compared to the data obtained from the first one hour. The highest TPC for TMAB was experienced by the control sample, which did not undergo any ozone treatment with 3.58×10^9 CFU/gram, followed by samples with a combination of duration - frequency of 2 minutes - 3 times, 3 minutes - twice, and 6 minutes – once with each TPC of aerobic bacteria mesophyll 2.24×10^9; 2.33×10^9; and 1.81×10^9 consecutively. Both in the first one hour and 168th hour sample that has the best ability to reduce TMAB are samples that undergo ozonation treatment with the longest duration and lowest frequency, which is sample sprayed once for 6 minutes. This trend shows that ozonation treatment in spinach samples is more effective in reducing TPC of TMAB when it is executed with combination of long duration and low frequency than short duration - high frequency. This is due to the potential loss of ozone during valve switching when twice and 3 times frequency were used, causing estimated total ozone exposure less than sample with combination of long duration and low frequency.

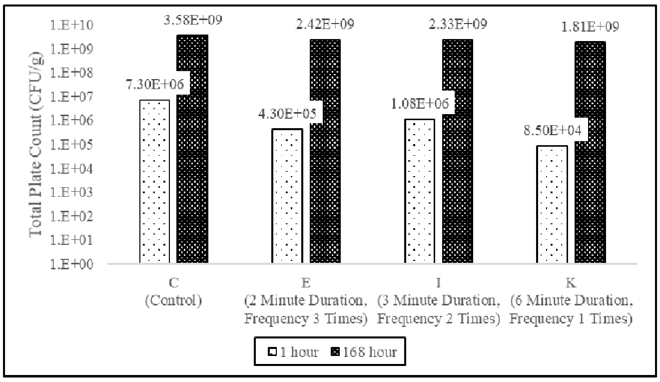

Fig. 15 The Effect of Duration-Frequency Combination towards TMAB during Storage Time

2) The Change of Vitamin C Content: Observing from the ability to reduce the rate of decomposition of vitamin C during 168 hours of storage, the sequence of the combination of duration - frequency from the best are sample I (duration 3 minutes - frequency twice), sample K (duration 6 minutes - frequency once), control, and sample E (duration 2 minutes - frequency 3 times). There is a significant difference between sample I and K compared to control sample and E sample. It can be seen that the combination of a longer duration with a smaller frequency of spraying can slow down the rate of vitamin C decomposition better than a combination of a short duration with a frequency of more than twice.

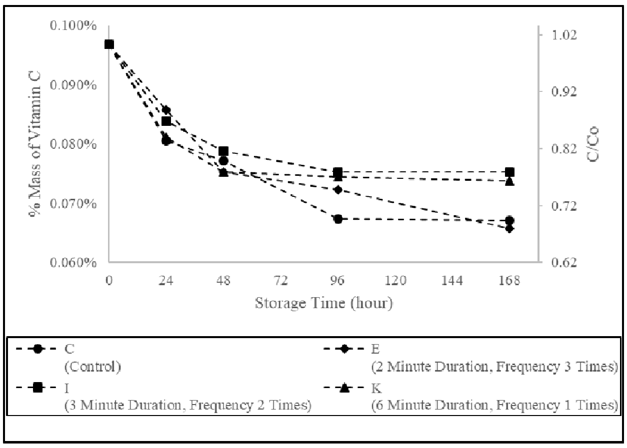

Fig. 16 The Effect of Duration-Frequency Combination towards Vitamin C Content during Storage Time

If this data is related to TPC results of aerobic mesophyll bacteria testing effect, visual changes, and odor changes, there is a similar observable trend. Besides, it can be concluded that the better the combination of the duration and frequency of ozone gas spraying in disinfecting bacteria, the slower the rate of respiration, the slower physiological changes such as nutrient loss, and the slower appearance of decay symptoms such as odor and discoloration start to happen. The smaller the number of bacteria means the less need for vitamins to be absorbed.

3) The Change of Mass: The sequence of mass change rate from the fastest are control sample, 6 minutes - once, 3 minutes - twice, and 2 minutes - 3 times. These results do not support data of mass changes obtained right after ozonation treatment, nor data of TMAB count, organoleptic feature, and vitamin C content. This can be resulted from simultaneous reactions between reactions that result in mass gain and mass loss so that mass changes that occur cannot be seen only as a result of water transpiration and nutrient decomposition which decreases the quality of spinach.

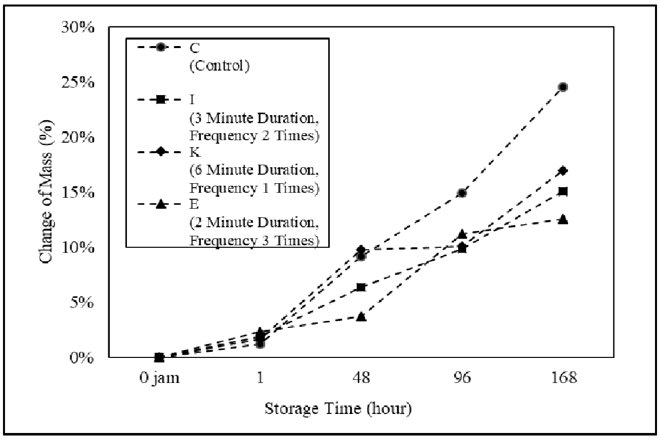

Fig. 17 The Effect of Duration-Frequency Combination towards Changes of Mass during Storage Time

4) The Change of Organoleptic Feature: After 7 days of storage period, it was observed that samples with various variations in duration and frequency combinations obtain higher color, odor, and texture score tests compared to control and they still meet the standard. The most optimum treatment in maintaining visual quality and odor is the combination of 6 minutes-once and for textures is 3 minutes-twice.

E. The Effect of Duration-Dosage Combination

The combination duration and dosage varied in this study were 2 minutes - 80.16 mg/hour, 3 minutes - 48.60 mg/hour, and 6 minutes - 30.72 mg/hour which are based on an estimated equivalent amount of ozone exposure. Fixed variables include spraying once spraying frequency, ozonation temperature of 28°C and storage temperature of 8°C. In fact, the amount of ozone exposure obtained with these variations is not equivalent as can be seen in Table 1.

TABLE I
TOTAL OZONE EXPOSURE FOR VARIOUS COMBINATION OF DOSAGE AND DURATION

Sample	Treatment			
	Dosage (mg/hr)	**Dosage (mg/min)**	**Duration (min)**	**Total Exposure (mg)**
D	80.16	1.336	2	2.672
G	48.60	0.81	3	2.430
K	30.72	0.512	6	3.072

1) The Change of TMAB: Figure 18 and Figure 19 show that TMAB reduction due to ozonation treatment by spraying ozone gas in spinach samples is more effective in reducing TMAB if it is done at smaller dosages and longer contact duration combination rather than combination of high dosage with short duration. This result is in line with the conclusion of [19], where the ozonation treatment of longer duration and lower concentration is more effective than its opposite. However, this can also be caused by the fact that K sample (duration of 6 minutes, dosage of 30.72 mg/hour) when calculated is also the sample with the highest total ozone exposure compared to other samples.

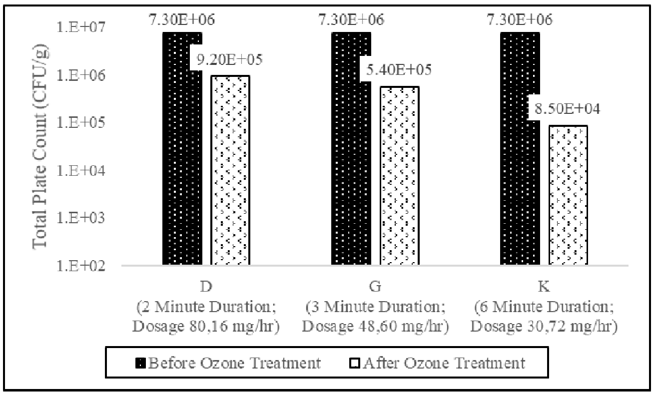

Fig. 18 The Effect of Duration-Dosage Combination towards TMAB

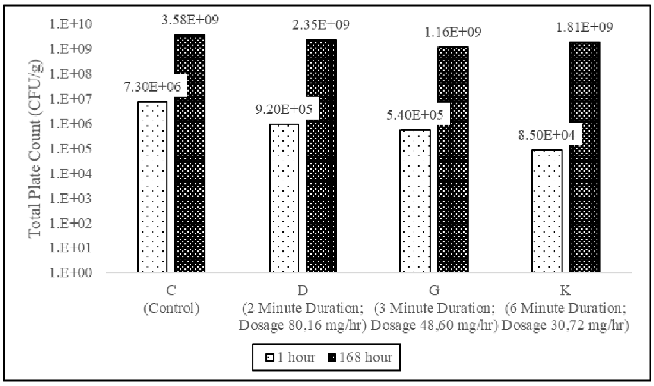

Fig. 19 The Effect of Duration-Dosage Combination towards TMAB during Storage Time

Both data from the first one hour and the 168th hour shows that the sample that have the worst bacterial deactivation are the ozonated samples with the combination of shortest duration and highest dosage which is 80.16 mg/hour for 2 minutes, meaning that ozone treatment with high dosage at short durations cannot be recommended. Through the results of this study, it can be indicated that the best duration of spinach samples in slowing infection and bacterial growth during storage is 3 minutes - 48.60 mg/hour.

2) The Change of Vitamin C Content: Observing Figure 20, the ability to reduce the rate of vitamin C decomposition during 168 hours of storage time, the sequence of duration-dosage combination from the best are sample G (duration 3 minutes - dosage 48.60 mg/hour), sample K (duration 6 minutes - dosage 30.72 mg/hour), control, and sample D (duration 2 minutes - dosage 80.16 mg/hour). There is a significant difference between the G and K samples compared to control and D samples. The sequence of the rate of vitamin C decomposition during 168 hours of storage. It can be seen that the combination of longer duration with smaller spraying dosage (such as a combination of 3 minutes - 48.60 mg/hour and duration of 6 minutes - frequency 30.72 mg/hour) can slow down the rate of decomposition of vitamin C better than the combination of short duration with high dosage (2 minutes - 80.16 mg/hour). Compared with the microbiology analysis results, the sample combination of longer duration with a smaller dosage of spraying is also a sample with a slower rate of increase in the number of mesophyll aerobic bacteria during storage.

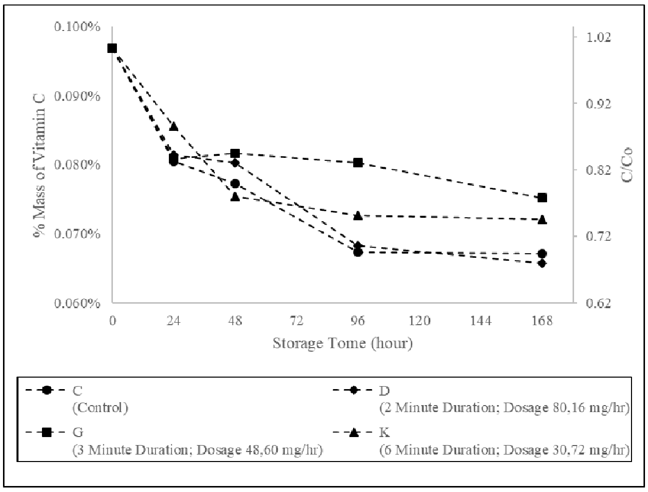

Fig. 20 The Effect of Duration-Dosage Combination towards Vitamin C Content during Storage Time

3) The Change of Mass: As can be seen in Figure 21, the rate of mass changes for 7 days storage sequence from the fastest are the control sample, 6 minutes - 30.72 mg/hour; 2 minutes - 80.16 mg/hour; and the best is the sample with a combination of the duration of 3 minutes – dosage of 48.60 mg/hour. If these results are related to texture score and vitamin C analysis results, the sample with the lowest percentage of mass loss during storage is also the sample with the best texture test score and the highest percentage of vitamin C content. Thus, ozonation treatment with this combination of duration and dosage produces the slowest respiration rate, causing a slowing of mass loss, nutrient loss, and morphological changes.

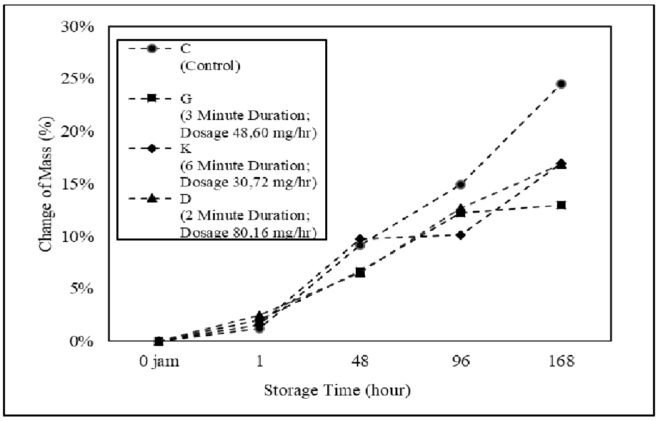

Fig. 21 The Effect of Duration-Dosage Combination towards Changes of Mass during Storage Time

4) The Change of Organoleptic Feature: After 7 days of storage, samples with various combinations of duration and dosage get a higher color, odor, and texture score test compared to the control sample, and they still meet the standard. The most optimum treatment in maintaining visual and odor quality is a combination of 6 minutes - 30, 72 mg/hour and for texture is 3 minutes - 48.60 mg/hour.

IV. Conclusion

Ozone gas spraying can effectively disinfect bacteria in spinach and extend the shelf life of spinach judging from its ability to reduce TMAB by 85.2 - 98.8%, slowing down the rate of decomposition of vitamin C and mass loss and preventing the emergence of organoleptic spoilage symptoms. Spraying ozone gas with a higher dosage cannot guarantee an increase in spinach quality in terms of the TMAB count, vitamin C content, mass changes, and organoleptic feature. Ozone gas spraying with a longer duration positively influences the quality of spinach during storage in terms of the TMAB count, vitamin C content, mass changes, and organoleptic feature. Ozone gas spraying with a frequency of more than once (twice and 3 times) is better in maintaining the quality of spinach during storage in terms of the TMAB count, vitamin C content, mass changes, and organoleptic feature. For the estimated equivalent total ozone exposure, the combination of longer duration - lower frequency and moderate duration - moderate dosage is better in maintaining the quality of spinach during the storage time in terms of the TMAB count, vitamin C content, mass changes, and organoleptic feature.

Acknowledgment

The authors are grateful for the financial support from the Directorate of Research and Community Service, Universitas Indonesia through Hibah PUTI Q1Q2, with series number NKB-0296/UN2.R3.1/HKP.05.00/2019.

References

[1] H. Karaca, and Y.S. Velioglu,” Effects of ozone and chlorine washes and subsequent cold storage on microbiological quality and shelf life of fresh parsley leaves,” *Food Science and Technology,* vol. 127, pp. 1-9, 2020.

[2] R. Yang, and G.B. Keding,” Nutritional contributions of important African indigenous vegetables,” In *African indigenous vegetables in urban agriculture*. C. M. Shackleton, M. W. Pasquini, and A. Drescher, Ed. London: Earthscan, 2009, pp. 105-143.

[3] Alfaena, “Proyeksi Konsumsi Buah dan Sayur Utama di Indonesia Tahun 2018-2022,” B. Sc. Thesis, Institut Pertanian Bogor, Indonesia, 2018.

[4] E. P. Wahyuni, “Mempelajari Karakteristik Pengeringan Bayam Hijau,” B. Sc. Thesis, Universitas Lampung, Indonesia, 2018.

[5] A. A. Kader, *Post-harvest technology of holticultural crops*, 3rd ed., Oakland: University of California, 2002.

[6] S. Shezi, L.S. Magwaza, A. Mditshwa, and S.Z. Tesfay,” Review. Changes in biochemistry of fresh produce in response to ozone postharvest treatment,” *Scientia Horticulturae*, vol. 269, pp.1-9, 2020.

[7] R. Aslam, M.S. Alam, and P.A. Saeed, “Sanitization Potential of Ozone and Its Role in Postharvest Quality Management of Fruits and Vegetables,” *Food Engineering Reviews,* vol. 12, pp. 48–67, 2020.

[8] E.F. Karamah and N. Wajdi, “Application of ozonated water to maintain the quality of chicken meat: effect of exposure time, temperature, and ozone concentration,” *E3S Web of Conferences,* vol. 67, no. 04044. 2018.

[9] E.F. Karamah, N. Ismaningtyas, and A.P. Ilmiyah. “The effect of exposure time and water replacement in the application of ozonated

water to maintain the quality of tuna". *IOP Conf. Ser.: Mater. Sci. Eng.* Vol, 509, no. 012092, 2019.
[10] A. Alexopoulos, S. Plessas, Y. Kourkoutas, C. Stefanis, S. Vavias, and C. Voidarou, "Experimental effect of ozone upon the microbial flora of commercially produced dairy fermented products," *International Journal of Food Microbiology,* vol. 246, pp. 5–11, 2017.
[11] F. Zhu. "Effect of ozone treatment on the quality of grain products," *Food Chemistry,* vol. 264, pp. 358–366, 2018.
[12] E.F. Karamah, F. Amalia, R. Ghaudenson, and S. Bismo, Disinfection of Escherichia coli Bacteria Using Combination of Ozonation and Hydrodynamic Cavitation Method with Venturi Injector," *International Journal on Advanced Science, Engineering and Information Technology*, vol. 8, no. 3, pp. 811-817, 2018.
[13] A. Baggio, · M. Marino, · N. Innocente, · M. Celotto, · and M. Maifreni, "Antimicrobial effect of oxidative technologies in food processing: an overview," *European Food Research and Technology*, vol. 246, pp. 669–692, 2020.
[14] R. Rachmawati, M.R. Defiani, and N. L. Suriani, "Pengaruh Suhu dan Lama Penyimpanan Terhadap Kandungan Vitamin C pada Cabai Rawit Putih," *Jurnal Biologi*, vol. XIII, no. 2, pp. 36 – 40, 2009.
[15] N. A. Buchmeier, S. J. Libby, Y. Xu, P. C. Loewen, J. Switala, and D. G. Guiney, "DNA repair is more important than catalase for Salmonella virulence in mice," *Journal of Clinical Investigation*, vol. 95, no. 3, pp. 1047 – 1053, 1995.
[16] N. K. Hunt and B. J. Marinas, "Inactivation of Escherichia coli with ozone: chemical and inactivation kinetics," *Water Research*, vol. 33, no. 11, pp. 2633 – 2641, 1999.
[17] S. Wani, J. Maker, J. Thompson, and J. Barnes, "Effect of Ozone Treatment on Inactivation of *Eschericia coli* and *Listeria* sp. on Spinach," *Agriculture*, vol. 5, pp. 155-169, 2015.
[18] D. Bermúdez-Aguirre, and G.V. Barbosa-Cánovas, "Disinfection of selected vegetables under nonthermal treatments: Chlorine, citric acid, ultraviolet light and ozone," *Food Control*, vol. 29, pp. 82–90, 2013.
[19] J.R. Junttila, S.I. Niemela and J. Hirn, "Minimum growth temperatures of *listeria monocytogenes* and non-haemolytic listeria," *Journal of Applied Bacteriology*, vol. 65, pp. 321-327, 1988.
[20] B. Maherani, · M. Harich, · S. Salmieri, · M. Lacroix, "Antibacterial Properties of Combined non-thermal treatments based on bioactive edible coating, ozonation, and gamma irradiation on ready-to-eat frozen green peppers: evaluation of their freshness and sensory qualities," *European Food Research and Technology,* vol. 245, pp. 1095–1111, 2019.
[21] A.M. Vettraino, V. Vinciguerra, G. Pacini, R. Forniti, V. Goffi, R. Botondi, "Gaseous Ozone as a Suitable Solution for Postharvest Chestnut Storage: Evaluation of Quality Parameter Trends," *Food and Bioprocess Technology*, vol. 13, pp. 187–193, 2020.
[22] L. Anderson, P. Koech, A. Plymale, E. Landorf, A. Konopka, F. Collart, M. Lipton, M. Romine, and A. Wright, "Live Cell Discovery of Microbial Vitamin Transport and Enzyme-Cofactor Interactions," *ACS Chemical Biology*, vol. 11, no. 2, pp. 345-354, 2015.
[23] A.J. Keutgen, and E. Pawelzik, "Influence of pre-harvest ozone exposure on quality of strawberry fruit under simulated retail condition," *Postharvest Biology and Technology*, vol. 49, no. 1, pp. 10 – 18, 2008.
[24] E. Porto, E.G.A. Filho, L.M.A. Silva, T.V. Fonteles, R.B.R. do Nascimento, F.A.N. Fernandesc, E. S. de Brito, and S. Rodrigues. "Ozone and plasma processing effect on green coconut water," *Food Research International*, vol. 131, pp. 1-9, 2020.
[25] L. Wang, X. Fan, K. Sokorai, and J. Sites, "Quality deterioration of grape tomato fruit during storage after treatments with gaseous ozone at conditions that significantly reduced populations of Salmonella," *Food Control*, vol. 103, pp. 9–20. 2019.
[26] G. Kaiser. (2019). Biology Libretexts: Factors that Influence Bacterial Growth. [Online]. Available: https://bio.libretexts.org/Bookshelves/Microbiology/.
[27] T. Sopandi, and Wardah, *Mikrobiologi Pangan – Teori dan Praktik.* Yogyakarta: ANDI, 2016.