The Effect of Gaseous Ozone Exposure and Storage Room Temperatures on Ethylene Production, Peel Colour, and Total Soluble Solid Content of Banana Fruit (*Musa acuminata*) During Storage

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Abstract
The exposure of gaseous ozone and storage room temperatures have a great potential to inhibit the production of ethylene during the ripening of climacteric fruits like banana. Furthermore, the inhibition of ethylene production is expected to be able to extend shelf life. Therefore, this study aims to evaluate the production of ethylene and quality attributes of fruit consisting of peel colour and total soluble solid content during storage, under the effect of the concentrations of variation gaseous ozone exposure and storage room temperatures. Some green mature bananas were sampled for the study, and an exposure of ozone gas concentrations of 0, 0.3, 0.4, and 0.5 ppm were applied to the samples daily and were then stored at 5, 15, and 27°C of storage room temperatures for 21 days. The production of ethylene was measured every three hours while peel colour and total soluble solids content were measured every day during storage. The result showed a significant interaction between storage time, ozone concentration, and storage temperature for ethylene production, *a*⁺ and *b*⁺ values of peel colour. For the total soluble solid content change, interaction occurred only between storage temperature and time.

1. Introduction
Banana is a popular and high marketing value fruit worldwide due to its nutrition and good taste. FAO data (2018), reported that it is the fourth most consumed product with global production reaching 113 million tons in 2016. It is a type of climacteric fruit that undergoes a postharvest ripening process characterized by a green phase followed by a burst in the production of ethylene and respiration rate that signals the beginning of the climacteric period until they reach the point of rot. This implies that banana has a short shelf life, and this affects its distribution and commercial value in the market.

Brat et al. [1] specifically explained that ethylene in climacteric fruit affects fruit maturity, physical appearance, hardness, taste, aroma, and total soluble solid content. However, its ripening process is
inhibited by a reduction in the concentration of ethylene [2]. The interest of various research is to reduce the concentration of ethylene after the harvesting period to increase the shelf life of climacteric fruit such as banana. Hernandez et al. [3] used potassium permanganate (KMnO4) as an ethylene absorber in climacteric fruit. However, the method of using chemical compounds in fruit is detrimental to human health when consumed continuously for a long period [4]. Furthermore, the use of potassium permanganate is only applicable on a small scale. Therefore, it is necessary to have postharvest handling to inhibit safe ethylene production that is useful on a larger scale production. Some treatments that have great potential in safely reducing ethylene production are gaseous ozone exposure [2] and low-temperature treatment [5]. The exposure of gaseous ozone on products reduces bacterial, viral, and pesticide contaminants such that the quality of food is better maintained [6]. The nature of ozone, which disappears quickly in air and water media, therefore it is safe to be used in the food industry [7]. This study aims to evaluate the production of ethylene, skin colour, and total soluble solid contents on banana ripening, under the effect of various gaseous ozone exposure concentrations and storage room temperatures. Studies that integrate the application of ozone with storage temperature for evaluating the quality of a product of an indigenous tropical banana are still a new phenomenon in the field of postharvest technology.

2. Experimental procedures

This study was arranged in a completely randomized design, factorial 4 x 3 with three replications. The first factor was the gaseous ozone exposure in four different concentrations, namely 0 (no exposure), 0.3, 0.4, and 0.5 ppm, while the second was the storage temperature consisted of 5, 15, and 27°C, where 27°C, which is the ambient or open room was considered as the control temperature. The investigated parameters were measured daily for 21 days storage period. To determine the effect of storage time on the data analysis, this measurement time would be considered as the main factor in the analysis.

Samples of kepok bananas (Musa acuminata) were harvested directly from a plantation at Kulon Progo (Yogyakarta, Indonesia) at 4 months after the blossom appears. After harvest, bananas were transported to the laboratory, washed, and selected based on uniform shape, size, colour, and free from pests and diseases to be used in the study. The selected bananas were randomly divided into three different sample groups. The first, second, and third were intended for the measurement of ethylene production, colour, and total soluble solid content, respectively.

Ozone gas was produced using an ozone generator (Ozonizer, model PX – 902). The capacity of production was determined by the iodometric method (International Ozone Association, Revised Standardized Procedure 001/96). The results showed that concentrations of 0.3, 0.4, and 0.5 ppm are achievable running ozone generators for 10, 15, and 20 minutes, respectively.

The samples were placed in a plastic container and then exposed to gaseous ozone according to the predetermined concentrations every day. After the predetermined ozone concentration was reached, the container was opened and the samples were stored for 21 days at three different temperatures as stated above.

The measurement of ethylene production was done using a 3.34L glass container equipped with a hole and ribbon stop filled with three bananas. Subsequently gas sampling was drawn and directly measured using portable ethylene gas analyzer (Korno, model GT-903-JM, measuring range 0 - 50 ppm) then flowed back into the container. There was a total of 12 containers for the measurement of ethylene production, which was carried out every three hours during storage.

The total soluble solids content was measured using a refractometer (Atago, model Pal-Alpha 3840, measurement range 0 - 85%) from the second sample group that was stored in an open plastic basket. Around 4 grams of banana sample was homogenized in a mortar and pestle, then the resulted liquid was poured inside the refractometer. Measurement was carried out every day during storage.

Peel colour was determined using a colour meter (Sucolor, model SC-10) expressed as L*, a*, and b*. The values were taken on three different surface parts of each sample. Furthermore, colour measurement was carried out daily during storage, and in this study, only a* and b* values were considered for analysis.
The collected data were subjected to three-way repeated measure analysis using IBM SPSS Statistics (version 25, SPSS Inc., USA) software. The sources of variation include the concentration of gaseous ozone exposure, storage time, and room temperature. While the mean comparisons were evaluated using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Ethylene Production

Ethylene is an attribute that affects the ripeness of bananas. Fig. 1 (a), (b), and (c) showed the production of ethylene for all samples of treatment variations at each storage temperature. In general, it appears that bananas without gaseous ozone exposure (0 ppm, control) had a higher ethylene production than those that are treated with ozone, especially for 15°C storage temperature. However, 27°C and 5°C had no significant difference. This indicated that the effect of ozone exposure on a banana is very much effective to reduce the production of ethylene when applied at around its chilling temperature.

![Ethylene Production Graphs](a) 5°C, (b) 15°C and (c) 27°C

The production of ethylene for bananas that are treated with ozone decreased dramatically shortly after being exposed to ozone gas, this was indicated by a very sharp repeated decline on the graph. Furthermore, the lowest ethylene production was generally owned by bananas, which were exposed to ozone gas with a concentration of 0.5 ppm, then 0.4 ppm, and 0.3 ppm. Meanwhile, storage room temperature of 5°C generally makes the lowest ethylene production, followed by 15°C and 27°C, respectively. At 27°C, ethylene production in some samples exceeded 50 ppm, such that the gauges are not suitable for its measurement. Based on the result of statistical analysis, there was significant interaction between "storage time and ozone exposure", "storage time and temperature", as well as "ozone exposure and storage temperature" (p<0.05). This implies that the effectiveness of ozone exposure on the reduction of ethylene production was influenced by both the storage time and temperature. Furthermore, means comparative analysis (DMRT) indicated that the lowest ethylene production was found for storage temperature of 5°C while the largest was for 27°C. Also, the values were significantly different among each temperature investigated (p<0.05). In addition, ethylene production consistently decreased along with decreasing storage temperatures. Wills and Golding [2], reported that storage room temperature had an influence on ethylene production. In this case, lower the storage room temperature characterizes lower ethylene production. However, 0.5 ppm ozone exposure gave the smallest ethylene production and was found to be significantly different with 0.3 ppm and control. There was also a trend that ethylene production decreased with increasing concentration of ozone. These results are consistent with Chen et al. [8], which revealed a decrease in ethylene production
in cantaloupes treated with ozone compared to control. Furthermore, it was explained that ozone has the potential to oxidize ethylene and this would be more significant when the concentration of ozone exposure was greater.

3.2. Peel Colour

![Graphs showing a* and b* values for different storage temperatures.](image)

**Fig. 2.** a* value of all samples during storage at storage room temperature of (a) 5°C, (b) 15°C and (c) 27°C

**Fig. 3.** b* value of all samples during storage at storage room temperature of (a) 5°C, (b) 15°C and (c) 27°C

Peel colour is the primary attribute of banana that influences consumer approval. Its changes during storage are presented in Fig. 2 (a), (b), and (c) as well as Fig. 3 (a), (b), and (c) for a* and b* values, respectively.

A negative value of a* (-0.84) at the beginning of storage confirmed that the sample was still green in visual appearance. Afterward, this value changed to positive along with the storage period, which indicates the reduction of greenness and entering the ripening period. However, the patterns of the changes and maximum values were very different among investigated storage temperatures. Statistical analysis indicated that significant interactions were found for both the three interactions (p<0.05), this indicated that the change of a* values depended on both concentrations of ozone, storage temperature, and time of measurement. A temperature of 15°C had the smallest a* and the largest was for 27°C (p<0.05). This also indicated that the temperature of 15°C created the slowest colour change or gave the
best capability in maintaining the green colour of the sample, while the fastest occurred at 27°C. Whereas, 5°C was unable to efficiently maintain green colour, which is probably due to stronger effect of temperature than ozone exposure treatments that cause chilling injury of the banana samples indicating by the dark colour of the peel. Zhu et al. [5], reported that bananas will experience chilling injury when stored at temperatures less than 13°C. In view of ozone concentration, it was a trend that as the concentration increased the mean values of \( a^* \) would also increase. Furthermore, 0.5 ppm gave the largest \( a^* \) value and significantly differed from other concentrations (p<0.05).

Meanwhile, the value of \( b^* \) gradually decreased along with storage time, this indicated that the samples tended to change from green to yellow and then to the dark colour. The samples were continuously monitored until they have deteriorated. Similarly, the patterns of the changes and maximum values of \( b^* \) vary significantly among investigated storage temperatures. Furthermore, the statistical analysis indicated significant interactions among the three (p<0.05). A larger positive \( b^* \) value indicated that banana samples maintain yellow colour better. However, decreasing or negative values indicated that the peel colour of banana had passed yellow and become dark or deteriorated. The largest positive mean \( b^* \) value was found for 15°C storage temperature and the lowest was at 5°C (p<0.05). This is consistent with the results found for \( a^* \) value and confirmed that storage temperature of 15°C was the best in maintaining banana peel colour. In addition, the trend of peel colour changes for both \( a^* \) and \( b^* \) values are consistent with the results reported by Adi et al. [9] in their study about the peel colour changes of banana during storage.

However, in view of ozone concentration, the largest mean \( b^* \) value was for the control and the smallest for 0.5 ppm (p<0.05). Furthermore, there was a trend that as the concentration of ozone increased \( b^* \) values decreased. It seemed that low concentration would be better in maintaining \( b^* \) value. This also implied that the application of ozone gas should carefully consider the concentration, duration, and method of application.

The visual investigation also found that bananas treated by gaseous ozone exposure had more dark spots and browning on their peels. As the concentration increased, dark spots and browning on samples were also increased. The same phenomenon was reported by Das et al. [10], that high concentrations of ozone gaseous exposure during storage caused an undesirable surface discoloration of their product (tomatoes). At higher concentrations, ozone gas reduces yellowing on product probably by the inhibition of chlorophyll degrading enzymes and/or the induction of antioxidants that protect chlorophyll. Meanwhile, Miller et al. [11] explained that enzymatic browning on samples causes colour alterations by the action of the browning-related enzymes (polyphenol oxidase and peroxidase). Furthermore, an inhibitory effect of ozone on these enzymes is probably due to the high oxidation potential of ozone gas. Then, produced browning, which greatly increases with ozone concentration and storage time.

3.3. Total Soluble Solid Content (TSSC)

TSSC during storage are presented in Fig. 4 (a), (b), and (c). TSSC for all treatment combinations was gradually increased over the storage period. However, their rate and maximum values were different. Furthermore, the increment trend of TSSC is consistent with Adi et al. [9], in the study of banana ripening at the ambient temperature and strawberry [12]. An increase in the banana TSSC was possibly due to enzymatic degradation of stored starch which was converted into sugars.
Fig. 4. TSSC of all samples during storage at storage room temperature of (a) 5°C, (b) 15°C and (c) 27°C

Statistical analysis showed that the interaction occurred only for storage time and temperature (p <0.05). This implies that the increase of TSSC during storage period depend solely on the storage temperature. Furthermore, as the storage temperatures decreased the TSSC values also decreased (p<0.05). This implied that in view of TSSC preservation, the lower the storage temperature the better TSSC would be maintained or the longer would be the shelf-life of the samples. Sholihati et al. [13] also reported the effect of temperature on TSSC of bananas fruit, where the lower storage temperature resulted in the smaller TSSC values. This was caused by the decreased rate of starch conversion to sugar due to inhibition of hydrolase enzyme activity at low temperatures.

4. Conclusion

The combination treatments of gaseous ozone exposure and storage room temperature had great potential in extending the shelf-life of bananas. Furthermore, a significant interaction between storage time, ozone concentration, and temperature was found for ethylene production, a* and b* values of peel colour. While for TSSC change, interaction occurred only between storage temperature and time. Moreover, ethylene production consistently decreased along with decreasing storage temperatures and increasing ozone concentration. The storage temperature of 15°C resulted in the slowest colour change or gave the best capability in maintaining the green colour of the sample. While 5°C was unable to maintain peel colour and caused chilling injury of the samples, although it had been treated using ozone gas. In addition, a decreasing storage temperature resulted in a decreased TSSC values. This implied that the lower the storage temperature, the longer the shelf-life of the samples. Conclusively, the treatment of ozone gas might create dark spots and browning on the peel, therefore, it was necessary to consider comprehensively for its application.

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