Original Paper

Evaluation of the Impact of Oxygen and Carbon Dioxide Atmospheres on Respiration Rate Measurement of Cherry Tomato

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Understanding the original respiration properties of each fresh produce is necessary to appropriately design controlled atmosphere storage and modified atmosphere packaging. The oxygen (O_2) and/or carbon dioxide (CO_2) concentrations of produce in gas-tight containers are often measured to achieve this. However, the measured respiration rate might be underestimated when the time at the start of measurement and/or the volume of the headspace of gas-tight containers are inappropriate; the condition of lower O_2 and/or higher CO_2 concentrations decreases the respiration rate. In this study, we investigated the effects of manipulated O_2 and CO_2 concentrations on the measured respiration rates of cherry tomato (*Solanum lycopersicum*) stored at 25 ºC, using two experiments examining the differences obtained with the closing time and volume of a gas-tight cylinder. The results demonstrated that the measured respiration rate of cherry tomatoes was underestimated by both lower O_2 and higher CO_2 atmospheres in gas-tight containers. These results help initiate a discussion regarding the appropriate enclosure time, volume of gas-tight containers, and/or samples used to measure fresh produce respiration rates in gas-tight containers.

Keywords: accuracy, controlled atmosphere storage, fresh produce, gas-tight container, modified atmosphere packaging

1. Introduction

The respiration of fresh produce continues during the post-harvest process with the consumption of respiratory substrates, such as sugars and organic acids[1),2)]. Therefore, desirable commercial qualities are often lost, and continuing respiration may increase food loss. Controlling post-harvest respiration in fresh produce is thus essential. To this end, many technologies related to the reduction of respiration in fresh produce during storage and distribution have been developed, including controlled atmosphere (CA) storage[3)] and modified atmosphere packaging (MAP)[4)]. For these technologies, lower oxygen (O_2) and/or higher carbon dioxide (CO_2) around the produce play an important role in the reducing respiration[5), 6)], along with temperature control. For the appropriate design of the CA and MAP, determining the original respiratory properties of each fresh produce is

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essential. To achieve this, the O_2 and/or CO_2 concentrations of the produce are often measured using a gas-tight container[7)-9)]. For this method, the sample of the produce is placed inside a gas-tight container for a certain time, and the O_2 and CO_2 concentrations in the headspace of the container are then measured. At this time, the O_2 and CO_2 concentrations inside the container are decreased and increased, respectively, compared with those at the beginning of the measurement. Considering the role of lower O_2 and higher CO_2 in the CA and MAP technologies, the measured respiration rate might be estimated to be low when the time during the start of measurement is too long or when the volume of the headspace of the container is too small compared to that of the samples; in such situations, the O_2 and CO_2 concentrations would be too low and too high, respectively.

In fact, an early study on MAP in broccoli suggested that changes in the O_2 and CO_2 concentrations inside the packaging during measurement affected the measured respiration value[10)]. However, few studies have focused on these concerns during measurements using closed containers. Therefore, in this study, we investigated the effects of different O_2 and CO_2 concentrations on respiration rate values using a gas-tight cylinder.

2. Materials and Methods

2.1. Measurement conditions of respiration

Ripe cherry tomatoes (*Solanum lycopersicum* cv. Unknown) with a color degree of 90% or higher, harvested in Kumamoto Prefecture, Japan, in May and June 2023 were used for this study. These tomatoes were purchased from a green grocer in Tokyo and delivered to Japan Women's University via private car with an internal temperature of ≈23 °C. The average mass (g) per fruit was 11.0 with 0.5 standard deviation. The fruit with temperature adjusted to approximately 25 °C was placed inside a gas-tight acrylic cylinder with a capacity of 977.6 ml (Custom made, Nihon Techno Service, Ibaraki, Japan). The holes in the cylinder were closed using plastic plugs and a rubber sheet (Fig. 1), and each cylinder was then closed. The initial gas inside the cylinder was air containing approximately 20.9% of O_2 and approximately 0.04% of CO_2. The fruit inside the closed cylinder was stored into an incubator (FCI-280G, AS ONE, Osaka, Japan) adjusted to 25 °C for gas measurement during and after the storage period. The O_2 and CO_2 concentrations inside each cylinder were measured using a portable O_2/CO_2 analyzer (CheckPoint3, MOCON Europe, Ringsted, Denmark). Based on previous studies[7), 8), 11)], the

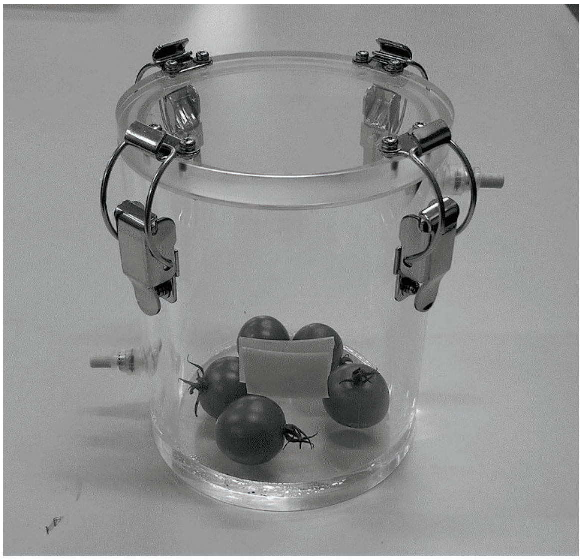

Fig. 1. Image of cherry tomatoes inside the acrylic gas-tight cylinder

production rate of CO_2 was assumed to be the respiration rate of the fruit within the measurement period and was calculated using the procedure described in the next section.

2.2. Calculation of respiration

According to the official database of food composition in Japan[12)], the water content of cherry tomatoes is 91%, and an early study determined the specific gravity of ripe tomatoes to be near 1.00[13)]. Therefore, in accordance with the report by Sato et al[14)], we calculated the headspace inside the cylinder containing the fruit (V_H, ml) by subtracting the total mass of the fruit (M_{CT}, g) inside the cylinder from the empty volume of the cylinder (977.6 ml).

When using the aforementioned portable O_2/CO_2 analyzer, the measurement values are indicated as percentages (%). Thus, we calculated the volume of CO_2 (V_{CO2}, ml) inside the cylinder using the following equations:

$$V_{CO2} = V_H \times 100^{-1} \text{ ...... Eq. 1}$$

The volume of the actual gas changed with the temperature difference. At the time of planning, we could evaluate gas concentrations under different temperature conditions. Therefore, according to Charles's law, the value of the CO_2 volume at 25 °C calculated using Eq. 2 was transformed into the value of that at 0 °C (273.15 K) (V_{CO2}') as follows.

$$V_{CO2}' = (V_{CO2} \times 273.15) \times (273.15 + 25)^{-1} \text{ ...... Eq. 2}$$

The respective mass (mg) and volume (ml) of CO_2 per mol at 0 °C and standard pressure (1.01×10^{-5} Pa) are 44000 and 22400. Therefore, the value obtained from Eq. 2 was transformed into a mass value (M_{CO2}, mg).

$$M_{CO2} = (44000 \times V_{CO2}') \times 22400^{-1} \text{ ...... Eq. 3}$$

The values obtained from Eq. 3 shows the volume of CO_2 produced per total mass (g) of the fruit sample. The volume of CO_2 produced per kg (1000 g) (M_{CO2}', mg) was calculated using the following equation:

$$M_{CO2}' = 1000 \times M_{CT}^{-1} \text{ ...... Eq. 4}$$

Finally, we divided the value obtained from Eq. 4 with the closing time of the cylinder. The respiration rate per hour (R_{SP}, mg CO_2 kg^{-1} h^{-1}) was then obtained from the following equation.

$$R_{SP} = M_{CO2}' \times h^{-1} \text{ ...... Eq. 5}$$

2.3. Experimental design to induce different O_2 and CO_2 conditions

2.3.1. Effect of differences in the closing time of the gas-tight cylinder (Experiment 1)

The purpose of Experiment 1 was to create conditions with different O_2 and CO_2 concentrations during different closing periods of the gas-tight cylinder containing the fruit. Approximately 100 g of cherry tomatoes were placed inside each cylinder, and then the cylinders were closed. We measured the O_2 and CO_2 concentrations at 22.5, 45.0, 67.5, and 90.3 hours. In this case, cylinders were prepared for each measurement period. Three cylinders were used as replicates for each measurement period.

2.3.2. Effect of differences of head space inside the gas-tight cylinder (Experiment 2)

The respiration rate of fresh produce after the harvest decreases naturally and gradually[15] during storage, except during the climacteric rise phenomenon[15, 16] or its occurrence stage[15]. For Experiment 1, although it seems that the cherry tomatoes had ripened and their climacteric stage had passed, the natural decrease in the respiration rate might have affected the measurement results. Overall, 90.3 hours were required to complete all the measurements. Thus, to minimize the effect of such a decrease, Experiment 2 was conducted to create conditions with different O_2 and CO_2 concentrations obtained from the different headspace volumes inside the cylinder containing the fruit. Approximately 52, 100, 200, and 300 g of cherry tomatoes were placed inside each cylinder, and the headspaces of the cylinders were 926, 878, 778, and 678 ml, respectively. After closing the cylinders, the O_2 and CO_2 concentrations inside the cylinders were measured at 22.5 hours.

In addition, using the same samples, we repeated the measurement at 22.5 h three more times to obtain data for discussing whether a natural decrease had occurred in Experiment 1. The following steps were repeated for this evaluation:

1) Each cylinder was closed for 22.5 h.
2) The O_2 and CO_2 concentrations inside the cylinder were measured.
3) Each cylinder was opened and the gas inside was replaced with air.
4) Each cylinder was closed for the next respiration measurement.

Three cylinders were used as replicates to assess each headspace.

2.4. Statistical analysis

For respiration data, Tukey’s test was conducted to determine the statistical difference among the data after confirming the homogeneity in variance using Bartlett’s or Levene's test. All statistical tests were performed using an add-in statistical software (Bell Curve for Excel version 4.04, Social Survey Research Information, Tokyo, Japan). The significance level for all tests was set as 0.05.

3. Results

3.1. Effect of differences in the closing time of the gas-tight cylinder (Experiment 1)

The O_2 and CO_2 concentrations inside the acrylic cylinder during each measurement period are listed in Table 1. The O_2 concentration inside the cylinder decreased with increasing closing time. However, the CO_2 concentration inside the cylinder increased with an increase in the closing time. When the cylinder was closed for 22.5, 45.0, 67.5, and 90.3 hours, the values of respiration rate (CO_2 mg kg^{-1} h^{-1}) were 35.7, 28.5, 26.0, and 22.8, respectively (Fig. 2). A significant difference was observed between the value at 22.5 hours and that at the times.

Table 1. O_2 and CO_2 concentrations inside each cylinder

Closing time for measurement (h)	O_2 concentration (%)	CO_2 concentration (%)
22.5	16.4 ± 0.4[z]	5.1 ± 0.4
45.0	13.1 ± 0.5	8.3 ± 0.4
67.5	9.8 ± 0.4	11.1 ± 0.3
90.3	7.3 ± 0.2	13.1 ± 0.3

[z]Average ± Standard deviation (n = 3).

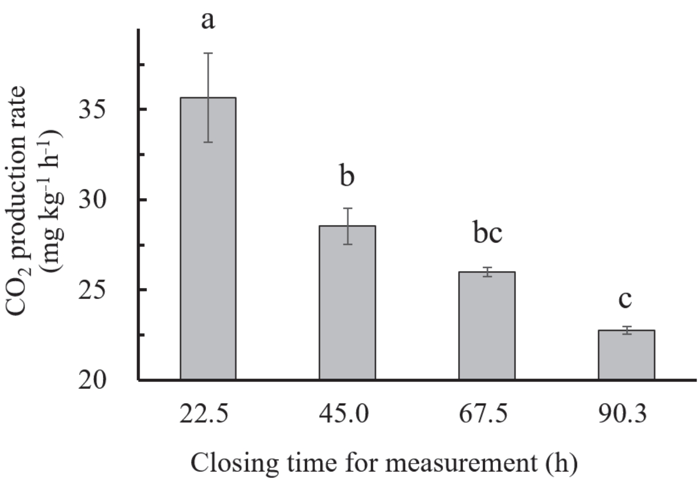

Fig. 2. Effect of differences in the closing time of the cylinder on the measured respiration rate value. Different small letters indicate significant differences at $p < 0.05$ using Tukey's test. Error bars show standard deviation (n = 3).

3.2. Effect of differences in head space inside the gas-tight cylinder (Experiment 2)

The O_2 concentration inside the cylinder decreased with decreasing headspace (Table 2). For example, the O_2 concentration inside a cylinder with a head space of 678 ml decreased to 65% from that with a head space of 926 ml. In contrast, the CO_2 concentration inside the cylinder increased with a reduction in the headspace. When the headspace was 678 ml, the value increased 5.4-fold compared to that when the headspace was 926 ml. The values of the respiration rate (mg CO_2 kg^{-1} h^{-1}) for the headspace of 926, 878, 778, and 678 ml were 36.9, 31.4, 28.4, and 25.2, respectively (**Fig. 3**). Thus, the respiration rate decreased linearly and significantly with a decrease in headspace, that is, with an increase in the mass of the sample inside the cylinder.

Table 2. O_2 and CO_2 concentrations inside each cylinder

Volume of head space (ml)^z	O_2 concentration (%)	CO_2 concentration (%)
926	18.6 ± 0.1^y	2.6 ± 0.1
878	16.7 ± 0.2	4.5 ± 0.2
778	11.9 ± 0.4	9.1 ± 0.4
678	6.6 ± 0.3	14.0 ± 0.2

^{z}Calculated using Eq. 1.

^{y}Average ± Standard deviation (n = 3).

The relationship between the O_2 and CO_2 concentrations and the respiration rate during repeated closing and opening of the cylinder is shown in **Table 3**. When the headspace of the cylinder was 926 ml, both the consumption of O_2 and the production of CO_2 decreased gradually during the investigation.

Following this tendency, the respiration rate was also decreased during the investigation; the value for three repetitions was 25% lower than that for no repetitions. A similar tendency was observed for a head space of 878 and 778 ml inside the cylinder. However, when the headspace of the cylinder was 678 ml, no relationship was observed between the repetitive opening and closing of the cylinder and the change in respiration during the investigation.

4. Discussion

In Experiment 1, when the O_2 and CO_2 concentrations were 13.1% or less and 8.3% or more, respectively (**Table 1**), the respiration rate of the fruit was decreased significantly (**Fig. 2**). For CA storage and MAP, lower O_2 and/or higher CO_2 concentrations around the produce are known to play a role in inhibiting respiration[5), 6)]. For example, modified atmosphere packaging with 5% O_2 and 5% CO_2 concentrations at 5 °C was effective for reducing the respiration of cherry tomatoes[17)]. Generally, for fresh produce, the effect of respiration reduction by the low O_2 and high CO_2 atmosphere is significant with the higher storage temperature[18)]; our experiments were carried out at 25 °C. Thus, for Experiment 1, the respiration rate of cherry tomatoes was suggested to be changed by the lower O_2 and higher CO_2 atmospheres owing to the difference in the closing time of the gas-tight cylinder. However, these results might include the effect of natural reduction in the respiration of the samples[15)].

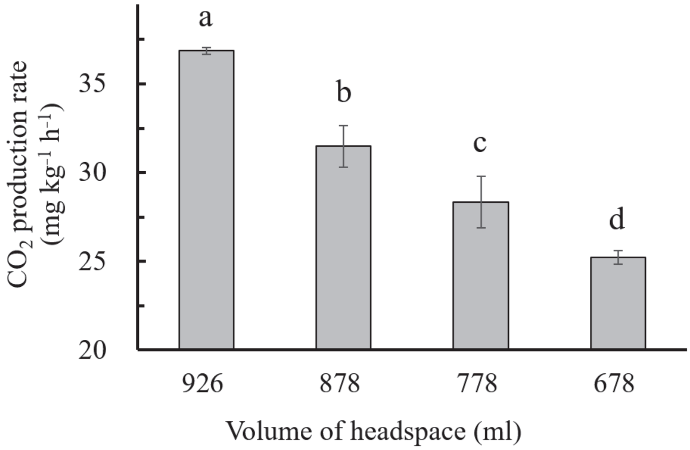

Fig. 3. Relationship between the head space and respiration rate.
Different small letters indicate significant differences at $p < 0.05$ using Tukey's test.
Error bars show standard deviation (n = 3).

Table 3. Change in O_2 and CO_2 concentrations inside each cylinder and in the respiration rate

Volume of headspace (ml)[z]	Repetition of measurement	O_2 concentration (%)	CO_2 concentration (%)	CO_2 production rate (mg kg^{-1} h^{-1})
926	-[y]	18.6 ± 0.1[x]	2.6 ± 0.1	36.9 ± 0.2
	1	18.9 ± 0.1	2.3 ± 0.0	33.0 ± 0.8
	2	19.1 ± 0.0*	2.1 ± 0.1*	29.0 ± 1.2*
	3	19.2 ± 0.0*	2.0 ± 0.1*	27.6 ± 1.2*
878	-	16.7 ± 0.2	4.5 ± 0.2	31.5 ± 1.2
	1	17.0 ± 0.2	4.2 ± 0.1	29.8 ± 0.8
	2	17.2 ± 0.2	3.9 ± 0.2	27.7 ± 0.9
	3	17.4 ± 0.0	3.8 ± 0.1	26.5 ± 0.3
778	-	11.9 ± 0.4	9.1 ± 0.4	28.4 ± 1.4
	1	12.4 ± 0.5	8.9 ± 0.6	27.8 ± 1.8
	2	12.9 ± 0.5	8.2 ± 0.6	25.7 ± 1.8
	3	13.2 ± 0.5	7.9 ± 0.5	24.6 ± 1.7
678	-	6.6 ± 0.3	14.0 ± 0.2	25.2 ± 0.4
	1	7.2 ± 0.4	14.5 ± 0.3	26.1 ± 0.3
	2	7.9 ± 0.1*	13.8 ± 0.0*	25.1 ± 0.0*
	3	7.9 ± 0.2*	14.1 ± 0.1*	25.5 ± 0.1*

[z]Calculated using Eq. 1.

[y]Same as the data shown in Table 2 and Fig. 3.

[x]Average ± standard deviation (n =3). However, asterisks show the results obtained from two replications due to the lack of data caused by the occurrence of rot.

Thus, for Experiment 2, we evaluated the respiration rate of cherry tomatoes with the same closing times but different headspace volumes and sample masses. As the headspace reduced with increasing mass of the sample, both the reduction of O_2 and increase of CO_2 were remarkable, and the respiration rate decreased linearly and significantly (**Table 2** and **Fig. 3**). Under such conditions, both reducing the headspace and increasing the mass of the sample was suggested to induce rapid O_2 reduction and CO_2 enhancement. Nevertheless, the results of this experiment also suggest that the respiration rate of cherry tomatoes was decreased by lower O_2 and higher CO_2 atmospheres.

An additional evaluation of Experiment 2 demonstrated that the natural decrease in respiration did not affect the measured value when both lower O_2 and higher CO_2 conditions were maintained during the closing of the cylinder (**Table 3**). This was attributed to a reduction in the consumption of respiratory substrates. Nevertheless, this result also suggests that the natural decrease in respiration observed in Experiment 1 was small or negligible in this study.

5. Conclusion

The results of this study demonstrated that the measured respiration rate of cherry tomatoes was underestimated by both lower O_2 and higher CO_2 atmospheres in gas-tight containers. This finding also suggested that the accuracy of the respiration measurement values might decrease if the closing time and/or volume of gas-tight containers or samples were inappropriate compared to the respiration rate of each type of fresh produce. Our next study will focus on determining the appropriate mass of samples, closing time, and volume of gas-tight containers.

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ミニトマトの呼吸測定における 酸素および二酸化炭素雰囲気の影響評価

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青果物の呼吸特性を把握するために、しばしば密閉容器を用いた呼吸速度の測定が行われる。しかし、青果物の呼吸速度は低酸素および高二酸化炭素環境では低下することから、密閉容器を用いた測定において容器の密閉時間や空隙の体積が不適切な場合には、そのような雰囲気が生じ、呼吸速度を過小評価してしまう可能性が考えられた。そこで本研究では、酸素と二酸化炭素濃度の違いが 25℃で貯蔵したミニトマトの呼吸速度の測定値に及ぼす影響を、容器の密閉時間と空隙の体積の違いに関する 2 つの実験により検証した。その結果、ミニトマトの呼吸速度は密閉容器内が低酸素および高二酸化炭素環境となった場合に、本来よりも低く見積もられる可能性が見出された。それらの結果は、密閉容器を用いた青果物の呼吸速度の測定における、最適な密閉時間および容器の体積またはサンプル重量の検証といった新たな議論の発端となる。

キーワード：精度、CA 貯蔵、青果物、気密性容器、MA 包装