Measuring System of the Respiration Rate of Fresh Produce under Continuous Changes in CO_2 or O_2 Concentration

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SUMMARY

A system for measuring the respiration rate of fresh agricultural produce under continuously changing gas concentration is designed. The respiration rates of eggplant under continuously increasing or decreasing of CO_2 or O_2, respectively, were measured using this system. An effective depression of the respiration with changes in CO_2 or O_2 concentration was observed. The result suggests that the system is well designed to measure the respiration rate of fresh produce under controlled atmosphere conditions.

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INTRODUCTION

It is well known that respiration is one of the most important metabolisms in post-harvested agricultural produce and a valuable index of the quality, and that control of the respiration rate should be the basis of freshness keeping [1]. A higher respiration rate of post-harvest produce leads to a deterioration in quality due to the degradation of their internal components such as starch, sugars, lipids and organic acids.

Storage of agricultural produces under a controlled atmosphere is one of the most effective techniques for their freshness keeping [2], and modified atmosphere packaging (MAP) is a very convenient storage technique. The quality of agricultural products in MAP depends on both the respiration activity of the produce itself and properties of its packaging plastic film such as its permeability. Most previous studies on MAP mentioned the relation between respiration rate and surrounding gas composition, and the results obtained were very useful to develop some functional plastic films [3]. In these studies, however, the respiration rate was measured under a statically stable modified atmosphere with a constant gas composition. From these data, therefore, it was not possible to discuss the quality at the initial stage immediately after packaging when the inner gas composition was continuously changing. In that initial stage, the inner gas composition changes dynamically depending on the balance between the respiration activity of the fresh produce and the permeability coefficient of using film for O_2 and CO_2.

We proposed to consider the physiological properties of fresh produce in the initial period until inner gas composition becomes clearly stable after packaging with film. We did this by analyzing the data for respiratory reactions under continuously changing in CO_2 or O_2 concentration. At first, we developed a simple system to measure the respiration rate under continuously changing O_2 and CO_2 concentrations, allowing us to discuss the respiration metabolism in MAP.

MATERIALS AND METHODS

Construction of measuring system

The outline of the system is illustrated in Fig.1. The system is constructed of two flow pathways, one of pure gas (CO_2 or N_2) pathway and another of normal air. Gases from both pathways are mixed together in a mixing chamber and flow into the sample chamber and out from an exhausting pipe connected to the sample chamber. A modified atmosphere with a desired gas composition is obtained by regulating the flow rates of pure and normal gases. As occasion demands, the flow rate of the mixed gas is measured by a flowmeter (Shimadzu DFM-1000) attached to the exhaust pipe.

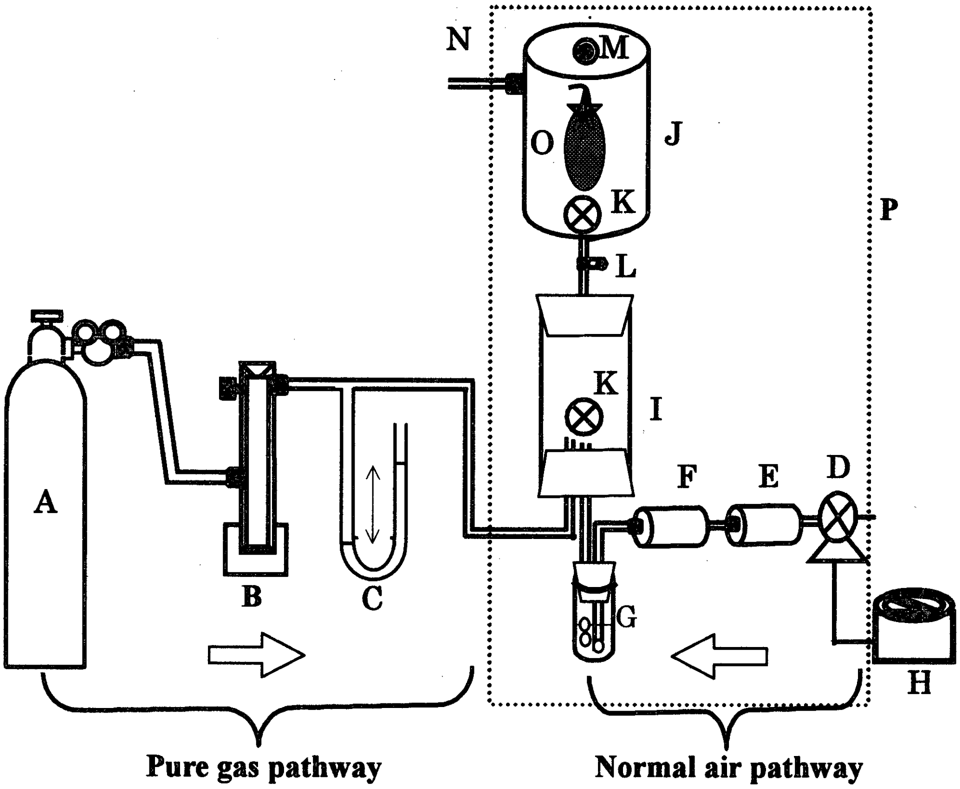

Fig.1 Illustration of measuring system of respiration rate of agricultural fresh produce under continuous changing in gas composition.
A; steel cylinder filled with pure gas (CO_2 or N_2), B; flow controller, C; U-shaped glass tube (arrow indicates U-shaped tube reading, see text), D; air pump, E; silica gel, F; NaOH, G; water bottle, H; voltage transformer, I; mixing chamber, J; sample chamber, K; fan, L; inlet sampling aperture, M; outlet sampling aperture, N; exhaust pipe, O; fresh produce (sample), P; incubator.

The mixing chamber is an acrylic pipe with a volume of 400 ml and internal diameter of 115 mm, and both sides are closed with rubber cocks. To mix pure gas (CO_2 or N_2) and normal air completely, an electric fan is placed inside. The sample chamber is also an acrylic pipe with a volume of 1845 ml and an electric fan at the bottom to homogenize the gas composition around the fresh produce. Gas sampling apertures are set at the lower and the upper positions of the sample chamber to extract the inlet and outlet gases, respectively.

a) Pure gas pathway: The pure gas pathway consists of a steel cylinder filled with CO_2 or N_2 (steel bomb), a flow controller and a U-shaped glass tube. The controller regulates the gas flow rate, which is measured with a U-shaped glass tube attached to the flow pathway tube. Ethyl alcohol (99.5%) is put in the glass tube, and we estimate the flow rate of pure gas by reading the distance (mm) between the upper and lower surfaces of the alcohol. Hereafter the distance is termed a 'U-shaped tube reading'. In experiments of continuously increasing CO_2 and decreasing O_2, we used CO_2 and N_2 in the pure gas pathway, respectively.

b) Normal air pathway: The normal air pathway consists of an air pump, two acrylic pipes (one with silica gel and another with NaOH), and a glass bottle filled with water. The flow rate of normal air is regulated by the power control of the air pump. We estimate the flow rate of normal air by reading the digital value on a voltage transformer connected to the pump. The silica gel and NaOH absorb moisture and CO_2 from the normal air, respectively. Glass bottle of water is used to keep the air in the sample chamber at a high constant humidity.

Measurement of respiration rate

The respiratory reaction of agricultural products is generally expressed by the following formula of chemical reactions; $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 674$ kcal. We measured the gas composition of extracted gas sampled from both apertures by a gas chromatograph (Shimadzu GC-14A) equipped with a thermal conductivity detector (TCD), and estimated the respiration rate from the difference between the inlet and outlet gas concentrations. Thus, the respiration rate of fresh produce was calculated by the following equation

$$R = \{|Cin\text{-}Cout| \cdot F \cdot M \cdot 10\}/\{0.082 \cdot T \cdot W\}$$

where R = respiration rate (mgCO_2 or O_2/kg/hr)

Cin = CO_2 or O_2 concentration (%) at inlet of sample chamber

Cout=CO_2 or O_2 concentration (%) at outlet of sample chamber

F = flow rate (l/hr)

M= molecular weight of CO_2 (44) of O_2 (32)

T = absolute temperature (K)

W = sample weight (kg)

In the present experiment, eggplant was used as the sample vegetable to investigate the effect of continuously changing CO_2 or O_2 concentration, because respiration is sensitive to the surrounding gas composition[4].

RESULTS

Adjustment of desired gas composition

In increasing CO_2 or decreasing O_2 experiments, modified mixed air was prepared by mixing CO_2 or N_2 with normal air. Since, in our experiments, it is necessary to adjust any desired gas composition rapidly in the sample chamber, we calibrated the relationship between the 'U-shaped tube reading' and inlet CO_2 concentration for the increasing CO_2 experiment, and that the between digital values of a voltage transformer and the inlet O_2 concentration for the decreasing O_2 experiment. Results obtained were shown in Figs.2 and 3. Calibration lines in Figs.2 and 3 were valuable only when the digital values of the voltage transformer and U-shaped tube reading, respectively, should be kept constant. Good linearity between them (in both figures, R^2 values were over 0.94) was obtained, showing that we can easily adjust the desired concentrations of CO_2 and O_2 in the range of 0-8% and 0-20%,

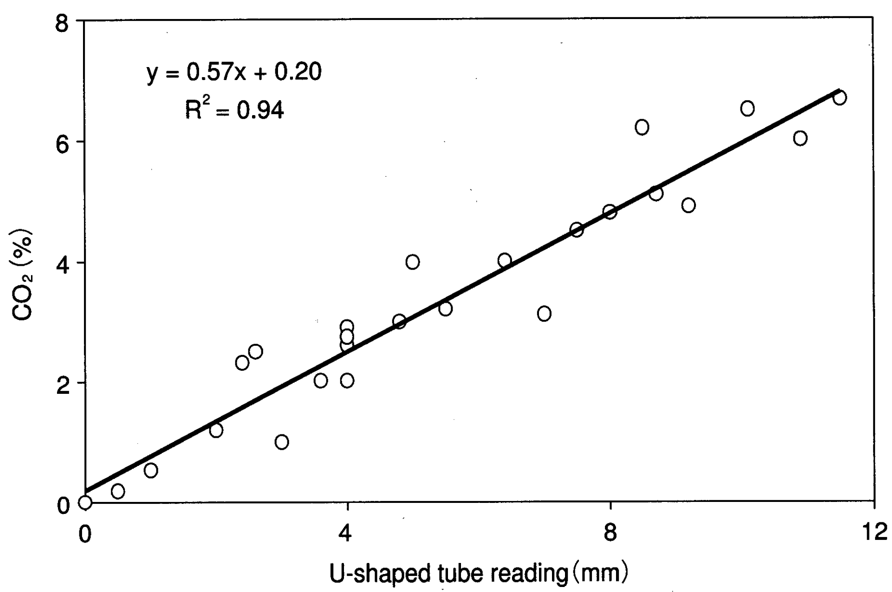

Fig. 2 Regulation of CO_2 concentration at inlet of sample chamber by flow controller, equipped in pure gas pathway, at 20 ℃.

Output of flow controller was estimated by U-shaped tube reading (see text). Digital value on voltage transformer connected to the pump in the normal air pathway was constant to be 20 V.

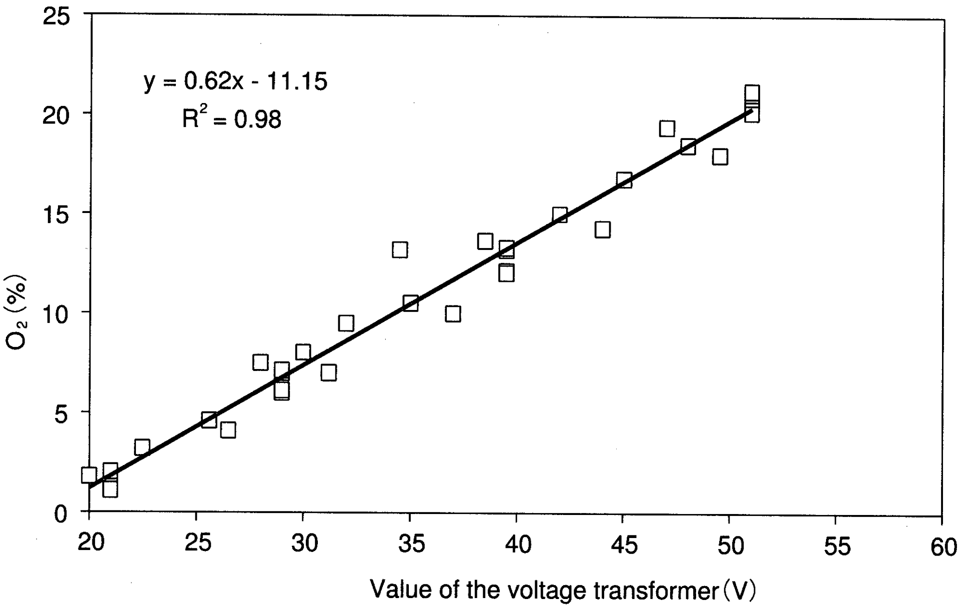

Fig. 3 Regulation of O_2 concentration at inlet of sample chamber by voltage transformer controlling output of air pump, equipped in the normal air pathway, at 20 ℃.
U-shaped tube reading for CO_2 supply in the pure gas pathway was kept constant to be 5 mm.

Table 1. Relation among inlet CO_2 concentration, U-shaped tube reading and flow rate of mixed gas.

average concentration of CO_2 at inlet (%)	U−shaped tube reading (mm)	flow rate (ml/min)
0 ± 0	0	92
2.61 ± 0.37	4	135
4.54 ±0.32	8	138
6.65 ± 0.42	12	140

Four different concentrations of CO_2 shown in the table were used in increasing CO_2 experiment. CO_2 concentrations (%) were expressed as means ± S.D. (n = 5). In the increasing CO_2 experiment, digital value of voltage transformer connected to the pump supplying normal air was kept constant to be 20 V.

Table 2. Relation among inlet O_2 concentration, voltage of transformer and flow rate of mixed gas.

average concentration of O_2 at inlet (%)	voltage of transformer (V)	flow rate (ml/min)
20.61 ± 0.56	51	200
12.63 ± 0.59	40	160
6.64 ± 0.55	29	145
1.69 ± 0.36	21	135

Four different concentrations of O_2 shown in the table were used in decreasing O_2 experiment. O_2 concentrations (%) were expressed as means ± S.D. (n = 5). In the decreasing CO_2 experiment, U-shaped tube reading was kept constant to be 5 mm, except for 20.6% O_2 condition where the value was zero (mm) because of normal air supplying.

respectively.

In the present study, we decided to measure respiration rates of eggplant at four different concentrations of CO_2 and O_2 in mixed air as shown in Table 1 and 2. In the increasing CO_2 experiment, respiration of eggplant was measured under CO_2 concentrations of 0, 2.6, 4.5 and 6.7 % and corresponding 'U-shaped tube readings' were 0, 4, 8 and 12 mm, respectively. The digital value of the voltage transformer was always kept constant at 20 V. In the decreasing O_2 experiment, N_2 gas was used to reduce the O_2 concentration in normal air, and the respiration was measured under O_2 concentrations of 20.6, 12.6, 6.6 and 1.7%, and the corresponding digital values of the voltage transformer were 51, 40, 29 and 21 V, respectively. The 'U-shaped tube reading' was adjusted to 5 mm except in the experiment for 20.6% of O_2 in normal air.

Gas mixing time inside the sample and mixing chamber

In our experiments, we had to change gas concentration continuously three times because we selected four concentration steps (Table 1 and 2). The respiration rate of eggplant has to be measured when the surrounding gas composition becomes homogeneous after adjusting gas concentration to the desired values by a flow controller (for the increasing CO_2 experiment) or a voltage transformer (for the decreasing O_2 experiment). Therefore, we investigated the time needed to reach a constant concentration in both mixing and sample chambers after changing the gas concentration. Fig.4 shows the time-course of inlet and outlet CO_2 concentrations after air modified with 4% of CO_2 is injected into the mixing chamber connected to the sample chamber. In this experiment, the flow rate of mixing air measured at the exhaust pipe was 140 ml/min. Inlet and outlet CO_2 concentrations correspond to the surrounding CO_2 concentration in mixing and sample chambers, respectively. The gas concentration in both chambers became constant within about 5 and 10 min, respectively, suggesting that we can measure the correct respiration rate of eggplant after 15 min since gas composition changes by regulation of pure or normal gas flow rates.

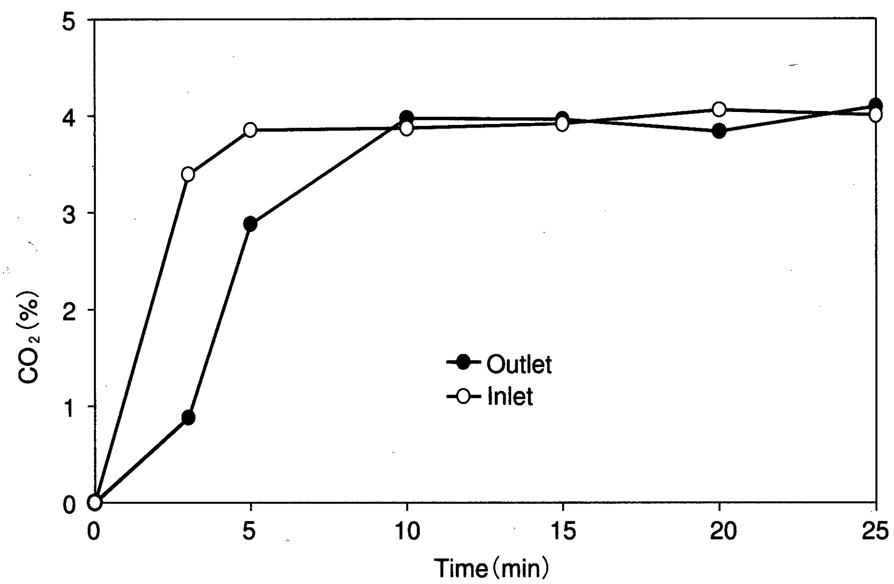

Fig. 4 Time-course of CO_2 concentration at inlet and outlet of sample chamber when modified air with 4% of CO_2 is injected into mixing chamber at 20 ℃.
The flow rate was 140 ml/min and U-shaped tube reading was 7 mm. Inlet and outlet CO_2 concentration represent the concentration in mixing and sample chamber, respectively.

Effect of continuously increasing CO_2 on the respiration rate of eggplant

Fig.5 shows the respiration rate of eggplant under gradually increasing CO_2 concentration (0→2.6→4.5→6.7% :

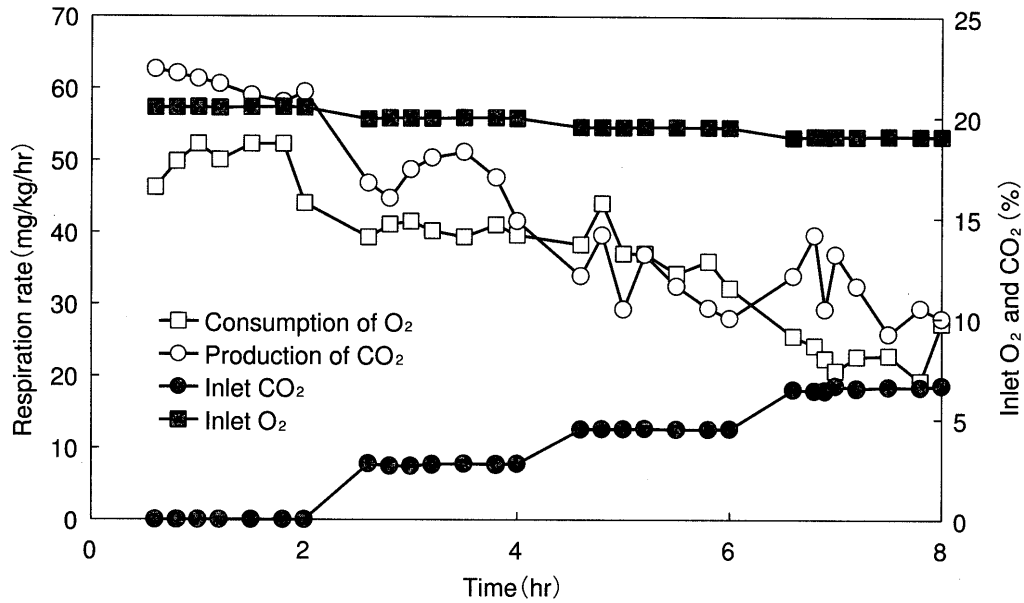

Fig. 5 Respiration rate of eggplant under continuously increasing CO_2 concentration at 20℃.
CO_2 production and O_2 consumption rates were measured at four different CO_2 concentrations shown in Table 1 (0, 2.6, 4.5 and 6.7%) and each step was 2 hr.

four steps) for 8 hr. In this experiment, we did not measure the respiration rate during interval between every step (about 30 min). O_2 concentration in mixed air was in the range of 20.6 - 19.2% but such a difference is negligible for the respiration of fresh produce. The O_2 consumption rate and CO_2 production rate at a CO_2 concentration of 0% (first step) were about 50 and 60 mg/kg/hr, respectively, and both rates decreased with increasing of inlet CO_2 concentration. At the final step with an inlet CO_2 concentration of 6.7%, both rates decreased to about 20 and 30 mg/kg/hr.

Effect of continuously decreasing O_2 on the respiration rate of eggplant

Fig.6 shows the respiration rate of eggplant under decreasing O_2 concentration (20.6 →12.6→6.6→1.7% : four

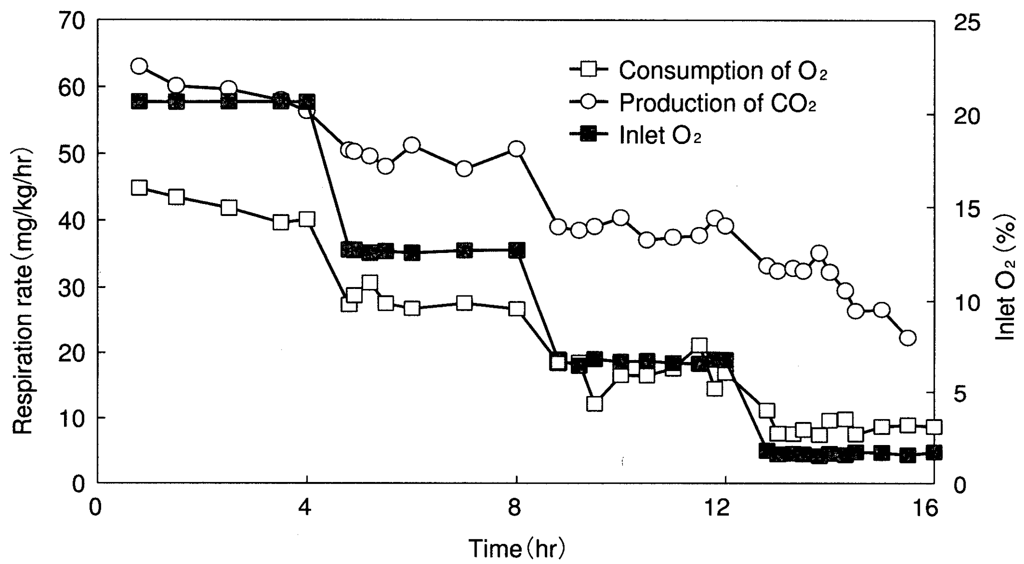

Fig. 6 Respiration rate of eggplant under continuously decreasing O_2 concentration at 20 ℃.
CO_2 production and O_2 consumption rates were measured at four different CO_2 concentrations shown in Table 2 (20.6, 12.6, 6.6 and 1.7%) and each step was 4 hr. Inlet CO_2 was always 0 % in the experiment.

steps) for 16 hr. In this experiment, we also did not measure the respiration rate interval between every step. The CO_2 concentration in mixed air was negligibly small because NaOH in the pathway absorbs CO_2 in normal air. The O_2 consumption rate and CO_2 production rates at the O_2 concentration of 20.6% (first step) were about 40 and 60 mg/kg/hr, respectively, and both rates decreased with decreasing inlet O_2 concentration. At the final step with inlet O_2 concentration of 1.7%, both rates decreased to about 8 and 28 mg/kg/hr.

DISCUSSION

Modified atmosphere packaging (MAP) is the usual method to keep quality of fresh produce. Its fundamental principal is the suppression of respiratory activity because the respiratory reaction accompanies with exhausting internal components such as respiratory substrates. Our purpose at present is an investigation of the effect of changing rates of gas concentration around fresh produce under MA conditions on the respiratory metabolism of the produces. We previously reported a method to measure the respiration rate of fresh produce packed in plastic film[5] using a computer simulation technique. In the present study, we tried to construct a system to measure the respiration rate of fresh produce directly under changes in the surrounding CO_2 and O_2 concentration, and we checked the availability of the system by measuring the respiration rate of eggplant in the range of CO_2 concentrations of 0 - 7% and O_2 concentrations of 2 - 20% .

Gas compositions of modified atmospheres used in the experiment were adjusted by mixing normal air and pure CO_2 or N_2 gas. Inlet gas composition in the sample chamber in the increasing CO_2 experiment was well regulated by adjusting the flow rate of CO_2 to the constant flow rate of normal air (Fig.2). The similarly the inlet gas composition in the decreasing O_2 experiment was regulated by adjusting the flow rate of normal air at a constant rate of N_2 (Fig.3). High linearity (R2 is over 0.94 in both Figs.2 and 3) shows that the system is well designed for our purpose. In the present study, regulated gas compositions were measured at four different concentrations, that is, 0, 2.6, 4.5 and 6.7% of CO_2 in the increasing experiment (Fig.5), and 20.6, 12.6, 6.6 and 1.7 % of O_2 in the decreasing O_2 experiment (Fig.6), and we only measured the respiration rate of eggplant. The inlet CO_2 and O_2 concentrations can be adjusted more precisely using this system which can also measure the respiration rate of other fruits and vegetables. Respiration rates of eggplant under normal air conditions shown in Figs.5 and 6 were not the same, and this difference between two data might be due to the variance the in eggplants used in the experiments.

Although decreasing O_2 concentration in the increasing CO_2 experiment is negligible in the present study, such a problem has to be solved in someway. Gas composition inside the sample chamber was regulated by mixing pure gas with normal air whose flow rate was controlled by the pump regulated by an electric transformer. Therefore, a very stable voltage supply to the pump is absolutely necessary to obtain the exact gas composition. Some automated systems for measuring respiration rates of fresh produce have been reported previously[6, 7]. However, such measuring apparatus were complicated to set up and very costly, and are out of general use. Moreover, it is important to take into consideration the boundary gas of fresh produce in experiments of respiration measurement because the boundary gas layer may affect the respiration of fresh produce in a gas-tight measuring system. Shimoi *et al.*[8] measured the respiration rate of purple passion fruits and Bower *et al.*[9] described a respiration measuring method for plant tissue, but they did not consider the boundary layer of the sample in describing the respiration rate. We used a fan inside the sample chamber to thoroughly mix up the inside gas whose composition relates to both the production of CO_2 and consumption of O_2 by fresh produce. Since our measuring system is easy to use and very adaptable, we can readily regulate the humidity around fresh produce by adjusting the flow amount of normal air through water in the glass bottle. The system has considerable promise as a way to measure respiration of fresh produce under a continuously changing gas concentration.

The respiration rate of fresh produce is known to decrease due to low O_2 and high CO_2 concentration in the storage atmosphere[10, 11]. Since CO_2 is the product of respiration, the suppression of CO_2 production in a respiratory reaction by self-produced CO_2 would be considered similar to the product-inhibition defined in an enzyme reaction.

In the present experiment, increasing CO_2 and decreasing O_2 rates were about 1%/hr and 1.5%/hr, respectively. Under MAP conditions in the actual distribution process of fresh agricultural produce, net increasing rates of CO_2 and/or decreasing rates of O_2 were different depending on the magnitude of the concentration gradient between inside and outside gas. Determining the effect of an increasing rate of CO_2 and a decreasing rate of O_2 on the respiration metabolism is our final goal and will be examined in our next experiment using this measuring system.

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二酸化炭素あるいは酸素濃度が連続的に変動する条件下における農産物の呼吸速度測定システム

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要　　約

ガス環境が連続的に変化する状況における農産物の呼吸速度を測定するシステムを構築した。ナス果実の呼吸速度を二酸化炭素あるいは酸素が連続的に増加あるいは減少するガス環境を創出して本測定システムで呼吸速度を測定したところガス環境に応じて効果的に呼吸速度が抑制されることを確認した。これらの結果は本測定システムが種々の農産物のCA環境下での呼吸測定に適用できることを示唆している。

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