Automated Microcomputer System for Measurement of $O_2$ Uptake, CO$_2$ Output, and C$_2$H$_4$ Evolution by Fruit and Vegetables

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Summary
An automated system was developed for measurement of the rates of $O_2$ uptake, CO$_2$ output, and C$_2$H$_4$ evolution by fruit and vegetables simultaneously by use of a microcomputer and three gas chromatographs. The microcomputer was also used to regulate sample temperature and prepare the gas mixtures of $O_2$, CO$_2$, N$_2$, and C$_2$H$_4$ in various concentrations and to regulate the flow rate. Changes in the rates of $O_2$ uptake, CO$_2$ output, and C$_2$H$_4$ evolution associated with the ripening of banana fruits and the wounding of winter squash fruits were measured to check the performance of the system. The stimulation of respiration by exogenous C$_2$H$_4$, and the inhibition of respiration and C$_2$H$_4$ evolution by a high concentration of CO$_2$ were measured with sweet potatoes and peaches, respectively. The rates of change of the gases corresponded to well-known physiological responses to given conditions of flow gas in every sample tested. CO$_2$ output from the sample materials could be measured even in the flow gas containing a high concentration of CO$_2$, up to 60%. C$_2$H$_4$ evolution could be measured directly in the presence of external C$_2$H$_4$. Analysis of the response of fruit and vegetables in gas metabolism, to various gaseous environments of the system was satisfactory.

Introduction
Respiration reflects the physiological condition of fruit and vegetables well. For this reason, it has been used as an index of the physiological response to the surrounding environment. Many methods have been devised for measurement of the respiration rate. Measurement of CO$_2$ output in a static condition has been favored over measurement of $O_2$ uptake because of its relative simplicity, but it is not feasible to measure CO$_2$ output under an atmosphere with a high concentration of CO$_2$. Another disadvantage of this method is that the internal concentration of CO$_2$ and C$_2$H$_4$ of fruit and vegetables sealed in a respiration chamber increases to a certain level, which may alter the respiration rates. It is, therefore, preferable to measure $O_2$ rate in continuous-flow conditions to evaluate the respiratory responses of fruit and vegetables to a given environment.

C$_2$H$_4$ has a strong effect on the physiology of fruit and vegetables, especially on respiration. It is important, therefore, to measure the rate of C$_2$H$_4$ evolution concomitantly with respiration and to identify the respiratory responses to exogenous C$_2$H$_4$ for prolonging the storage life of harvested horticultural crops.

Although we have already much information concerning the response of fruit and vegetables by respiration and C$_2$H$_4$ evolution to the gaseous environment, we are now re-examining the effects of the environment such as fluctuating temperature, high concentration of CO$_2$, low concentration of $O_2$, and existing C$_2$H$_4$, on the gas metabolism, in relation to the present Japanese commercial distribution system. For these studies we reported a tentative computer system for measuring respiration by fruit and vegetables (5). Since then we improved the system and obtained several new findings. Prior to reporting these results, this paper describes a method for measuring $O_2$ uptake, and CO$_2$ and C$_2$H$_4$ output by fruit and vegetables.
vegetables in an atmosphere which contains a significant amount of any of these gases.

**Materials and Methods**

**Gas flow system**

The diagram of the gas flow and analysis system is shown in Fig. 1. All the controllers, valves and temperature of incubators were controlled through a microcomputer. The flow rates of O\(_2\), CO\(_2\), and N\(_2\) from each of the high-pressure gas cylinders were regulated with an electrical flow-rate controller (Model SECU-1, STEC Inc.) to obtain the desired gas mixture. The pressure of mixed gas was reduced to 3 atm/cm\(^2\) with a precision-type pressure regulator (Model 5330, Kojima Flow Instrument Co.). The stream of mixed gas was diverted to the desired number of lines and the flow of each was regulated with a precision needle valve (Model 2203, Kojima). The stream of mixed gas was diverted to the desired number of lines and the flow of each was regulated with a precision needle valve (Model 2203, Kojima). The gas stream of each line was controlled to 100 ml/min, humidified by water, passed through a 6 mm \(\times\) 4 m copper tubing in an incubation chamber for thermal equilibration and then through the sample container maintained in the incubation chamber. A three-way solenoid valve was used to direct the sample effluent gas to waste or on to the main sampling loops (Loops 1 and 2) that were attached to the rotating stepping valve (Model MSG-4, Shimadzu Corp.). The volumes of loops 1 and 2 were 1 ml and 5 ml, respectively. Flow from the rotating valves was directed to the gas analysis columns (COL-1 and COL-3) in the gas chromatograph. All flow lines were connected with vinyl pipe with an inside diameter of 6 mm. For gas streams containing a low level of ethylene, the mixed gas was supplied directly from a gas cylinder containing appropriately mixed gases.

**Gas analysis**

The concentrations of O\(_2\), N\(_2\), and CO\(_2\) in the sample gas were measured by use of two gas chromatographs (GC) equipped with a thermal conductivity detector (GC-1, GC-2; both Model GC-8 A, Shimadzu). The GCs had five columns in total (Fig. 2); two of porapak Q (COL-1, COL-2), one of molecular sieve 5 A (COL-3), and two for reference (COL-4, COL-5). At the beginning of analysis, the gas flowed directly from COL-1 to COL-3. As soon as O\(_2\) and N\(_2\) eluted from COL-1, the column exchange valve directed the flow COL-1 to COL-2 for separation of CO\(_2\) and water. Molecular sieve 5 A in COL-3 separated O\(_2\) and N\(_2\) and is subject to degradation by CO\(_2\) and water (3). C\(_2\)H\(_4\) was analyzed on an alumina column in the third GC equipped with a flame ionization detector (Shimadzu, Model GC-6 AM).

**Hardware**

A 16-bit microcomputer (Model PC-9801m2, NEC Co., Ltd.) was used in the system. Handmade circuits for controlling temperature, solenoid valves, and stepping motors were set to the I/O port of the computer. Temperatures...
were controlled by operation of a heater only for the period necessary by a signal from the computer; a cooler was continuously operated. The stepping motor was used to rotate the shafts of the gas samplers and column exchanger because the rotation of the shaft had to be accomplished quickly and precisely. A large scale integrated circuit for the exclusive use of the stepping motor (PPMC-101 C, Ampere Co.) was selected for above reason.

Buffer amplifiers for matching the electrical impedance and amplifying the output voltage were set between the data logger and each GC. The gas flow rate controller was commanded from the computer through RS-232 C connection.

**Software**

The output voltage of the GC was read at the rate of 60 times per second from immediately before the beginning of each chromatographic peak until passage through the maximum point. The differences in the highest and the lowest values of the peaks were calculated to find the concentrations of \( \text{O}_2 \) and \( \text{CO}_2 \) in the injected gas based on calibration curves. At that time, because \( \text{N}_2 \) is probably not metabolized by fruit and vegetables, \( \text{N}_2 \) was used as an internal standard. As shown in Table 1, the ratios of \( \text{O}_2 \) and \( \text{CO}_2 \) to \( \text{N}_2 \) on chromatograms were more stable than the direct reading of each peak height. Rates of \( \text{O}_2 \) uptake and \( \text{CO}_2 \) output by the respiration of fruit and vegetables were computed from the differences between the concentration of \( \text{O}_2 \) or \( \text{CO}_2 \) in the original flow stream and that in the respired gas. The rate of \( \text{C}_2\text{H}_4 \) evolution was measured in the same way except that the internal standard of \( \text{N}_2 \) was not used. Compensation was made for changes in the volume of the injected gas caused by fluctuations in the room temperature by the computer, but not for fluctuations in atmospheric pressure. Temperature was controlled as described previously (5). The program was written in BASIC, but executed after partial compilation into a machine language to provide for rapid execution. In particular, the stepping motor for the column exchange had to receive a command to rotate at the expected time calculated from the injection time, within an error of 0.1 seconds, to provide for precise gas analysis.

**Running test with fruit and vegetables**

Tests of the system were made by measurement of well-known aspects of gas metabolism of fruit and vegetables: the respiration and

![Fig. 2. Gas analysis system with column-exchange technique. The gas flows can be changed from paths shown by the solid lines to those of the dotted lines in the GS and CE by the turning of the rotating shafts. Labels indicate: He, carrier gas (He) inlet for GC; GS, gas sampler; CE, column exchanger; GC, gas chromatograph; D, thermal conductivity detector; and COLs 1 to 5, gas analysis column (1: 3 mm \( \times \) 2 m Porapak Q, 2: 3 mm \( \times \) 1 m Porapak Q, 3: 3 mm \( \times \) 3 m Molecular Sieve 5 A, 4 and 5: reference).](image-url)

**Table 1. Stability in the measurement of \( \text{O}_2 \) and \( \text{CO}_2 \) concentrations in flow gas consisting of 20% \( \text{O}_2 \) + 40% \( \text{N}_2 \) with the internal standard technique of \( \text{N}_2 \).**

<table>
<thead>
<tr>
<th>Gas component</th>
<th>Peak height in voltage (mV)</th>
<th>Ratio to ( \text{N}_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CO}_2 )</td>
<td>2926.6 ( \pm ) 10.7</td>
<td>1.2189 ( \pm ) 0.0076</td>
</tr>
<tr>
<td>( \text{O}_2 )</td>
<td>1653.6 ( \pm ) 4.2</td>
<td>0.6887 ( \pm ) 0.0002</td>
</tr>
<tr>
<td>( \text{N}_2 )</td>
<td>2401.2 ( \pm ) 6.6</td>
<td>1.0000 ( \pm ) 0.0000</td>
</tr>
</tbody>
</table>

\( ^2 \) Differences in the highest and the lowest values of the chromatographic peaks.  
\( ^7 \) Means of 12 replications of the measurement at 1-h intervals \( \pm \) SE.
C₂H₄ evolution associated with the onset of ripening in bananas, the synthesis of C₂H₄ caused by the cutting of winter squashes, the respiration response by sweet potatoes to exogenous C₂H₄, and the responses in respiration and C₂H₄ evolution by peaches to a high concentration of CO₂. All plant materials were purchased from a market.

Results and Discussion

Chromatographic traces of five components, C₂H₄, CO₂, O₂, N₂, and H₂O, in the sample gas are shown in Fig. 3. Measurements of the rates of C₂H₄ evolution, O₂ uptake, and CO₂ output were stable when the sampled gas was injected every five minutes, because it was not necessary to measure H₂O, which had the longest retention time with slight tailing.

Bananas are a typical climacteric fruit, with a sharp increase in C₂H₄ evolution at the onset of the climacteric (1). This phenomenon was clearly demonstrated by this system (Fig. 4). C₂H₄ evolution was already detected at a low level at the start of measurement, because the bananas had their preclimacteric life span shortened by exposure to a known concentration of C₂H₄ for a given period, as calculated from a formula published elsewhere (6), to obtain results within a shorter period. C₂H₄ evolution increased sharply after 11 h, with the highest level at 16 h, and then decreased sharply. The quantity of O₂ uptake agreed well with that of CO₂ output, both showing a typical climacteric rise with the highest value at 35 h.

The evolution of C₂H₄ by banana fruit during exposure to exogenous C₂H₄ at the concentration of 10 ppm at 25°C is shown in Fig. 5 with the onset of the respiratory climacteric. Our system performed well in measuring the endogenous C₂H₄ directly even in the presence of external C₂H₄, although propylene has been favored as a substitute gas for external C₂H₄ (9). It was of interest that the pattern of C₂H₄ evolution during exposure to exogenous C₂H₄ resembled patterns obtained in air.

Figure 6 shows the changes in the rate of C₂H₄ evolution in winter squash fruits caused by their being cut into eight equal parts longitudinally. C₂H₄ began to evolve about 2 h

![Fig. 3. Gas chromatographic traces of O₂, CO₂, and C₂H₄ in the system. The traces show the gas analyses from the first cycle to the second. Arrows indicate: A, injection of the sample gas; B, change of the connection of the column COL-1 from COL-3 to COL-2; C, return of the connection of the COL-1 column to the original.](image)

![Fig. 4. Changes in the rates of O₂ uptake, CO₂ output, and C₂H₄ evolution during the onset of ripening in banana fruit at 25°C.](image)

![Fig. 5. Measurement of C₂H₄ evolution in banana fruit at 25°C under the condition of external C₂H₄ exposure at 10 ppm.](image)
after cutting, peaking at 11 h, and then decreasing toward the initial level. The rates of O2 uptake and CO2 output also increased together with the evolution of C2H4. This C2H4 associated with cutting is the “wound C2H4” reported by Hyodo et al. (4).

Figure 7 shows the respiratory reaction of sweet potatoes to exogenous C2H4 at the concentration of 100 ppm at 30°C. The rates of O2 uptake and CO2 output greatly increased after C2H4 exposure, reaching a constant level. When C2H4 was removed, the respiration rate both in terms of O2 uptake and CO2 output decreased sharply to the original level. These reactions of sweet potatoes to exogenous C2H4 resembled those of potatoes (9).

These results showed that the system monitored the gas metabolism of intact fruit and vegetables accurately when air or an air-like atmosphere was used as a flow gas. We then examined whether measurement was possible in a high concentration of CO2 as well. The results in 40% CO2 + 20% O2 + 40% N2 are shown in Fig. 8 for peach fruits. High CO2 strongly inhibited both O2 uptake and CO2 output. The rate of C2H4 evolution was also greatly inhibited with a reduction to almost zero. The results indicated that the measurements were possible even in a high concentration of CO2. However, the measurement of CO2 output was slightly unstable when the CO2 in the flow gas was 60% or higher. The assay of O2 uptake was stable even with CO2 at 80% or more.

The ability of the system to regulate temperature was described previously (5), so the results are not shown here.

In conclusion, the performance of the system in measuring the rates of O2 uptake, CO2 output, and C2H4 evolution by fruit and vegetables simultaneously and automatically was satisfactory. Similar devices have been described by several workers. An automated computer system for measuring the rates of CO2 output and C2H4 evolution in harvested horticultural crops was developed by Watada and Massie (12) using a gas chromatograph fitted with two types of detector, and also developed by Lee (7) using an infrared CO2 analyser in combination with a gas chromatograph. A paramagnetic O2 analyser has been used to measure O2 uptake by Young and Biale (10,11) with the assay done under 10% CO2 conditions in various kinds of fruit. Lougheed and Franklin (8) report on the use of an infrared CO2 analyser for the measurement of respira-
tion, and Dilley and Dewey (2) use an infrared CO₂ analyser in combination with a paramagnetic O₂ analyser. There have been no reports of a device like our system with the ability to measure O₂ uptake, CO₂ output, and C₂H₄ evolution simultaneously even under an atmosphere containing a significant amount of any of these gases. Our system may be too expensive and complicated. Probably a more suitable device must be developed. Our purpose, however, is not to recommend the system to other researchers, but to describe the precision of the system prior to reporting the results obtained. Measurement of the metabolism of three gases is essential to identify the most appropriate handling for harvested horticultural products.

Literature Cited