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Article

Various Cultivars of Citrus Fruits: Effects of Construction on Gas Diffusion Resistance and Internal Gas Concentration of Oxygen and Carbon Dioxide

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Abstract: Various cultivars of citrus fruits have unique constructions, such as thick outer skin. These constructions generate gas diffusion resistance between the atmosphere and the fruit, which can limit the gas exchange of O_2 and CO_2.This has not been sufficiently investigated. This study on seven cultivars of citrus fruit firstly aimed to investigate gas diffusion resistance utilizing the ethane efflux method; secondly, this study aimed to investigate the internal gas concentration of O_2 and CO_2. As a result, a cultivar of citrus fruit with slimmer outer skin thickness had lower resistance. For the internal gas, a high CO_2 concentration in comparison with the atmosphere was observed even in the fruits with the minimum resistance, and no considerable difference was observed among all cultivars, regardless of the gas diffusion resistance value. However, when the fruits were stored at 25 °C for 2 weeks, CO_2 gas concentration tended to increase and O_2 gas concentration tended to decrease, with an increase in the resistance value. Therefore, when the respiration of citrus fruits is activated at ambient temperature, the self-control system of internal gas concentration can be driven to suppress the respiration which was induced by gas diffusion resistance generated from their construction.

Keywords: citrus fruit; gas diffusion resistance; respiration; internal gas concentration

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1. Introduction

Respiration in citrus fruit after harvest consumes oxygen and produces carbon dioxide, the respiration reaction of which is conducted in the cell [1,2]. In the results, a decrease in the oxygen concentration and an increase in the carbon dioxide concentration are indicated in the cell. Since gas diffusion between the outside and inside of the fruit is driven by the concentration gradient, as indicated by Fick's first law of diffusion, oxygen diffuses from the outside to the inside of the fruit, and carbon dioxide diffuses from the inside to the outside [1,3–7]. However, gas diffusion is resisted by the constructions of the plant tissue of the fruit, such as the outer skin and inner skin of the fruit [7–12]. Thus, the internal oxygen and carbon dioxide gas concentrations are low and high, respectively, compared to the atmosphere, because oxygen diffusion from the outside to the inside and carbon dioxide from the inside to the outside can be suppressed by the resistance generated from the construction of the plant tissue [3,5,7,13–17]. Therefore, gas diffusion resistance is an important factor in understanding the internal gas component of the fruit and controlling the respiration of the fruit [1].

Various cultivars of citrus fruit, which are cultivated in Japan with more than a hundred varieties, have unique structures such as thick or slim outer skin. The outer skin in contact with the atmosphere may play an important role in resistance to gas diffusion between the outside and the inside of the fruit [1,18]. However, the relationship between the thickness of the citrus fruit skin and gas diffusion resistance is unclear. Additionally, the number of stomata on the surface of the outer skin and the void space and more specific constructions may affect gas diffusion resistance [7,8,12,15,17,19,20]. For example, the gas diffusion resistance of fruits was increased by coating the surface of the fruits with wax, even for a thickness of ca. 0.8×10^{-2} mm [21]. In citrus fruits, wax coating on the opening stomata limited CO_2 and O_2 gas, since the opening of the stomata can be the primary route of gas transportation [22]. In the study of Xiao et al., utilizing tomato fruit, it was indicated that gas diffusion inside the fruit was related to the intercellular void space and stomata [23]. Gas diffusion in apples seems to be related to the intercellular void space [24]. However, it is unclear whether the specific constructions of various citrus fruits affect the internal gas composition related to respiration.

In this study, gas diffusion resistance was investigated using a modified ethane efflux method and fruit tissue construction for seven cultivars of citrus fruit. Additionally, the internal oxygen and carbon dioxide gas concentrations were investigated before and after storage at 5 and 25 °C to clarify the effect of gas diffusion resistance on the internal gas components.

2. Materials and Methods

2.1. Plant Material

The citrus fruits used in this study were *Citrus iyo "Miyauchi iyokan"*, *Citrus unshu "Nankan No.20"*, and *Citrus spp. "Ehime Kashi No.28"*, *"Harehime"*, *"Kanpei"*, *"Setoka"*, and *"Shiranuhi"*. Seven cultivars of citrus fruits cultivated in Ehime were purchased in retail locations and stored at 10 °C until the experiment and were utilized for the experiment within a week.

2.2. Measuring Gas Diffusion Resistance and Construction Characteristics for Cultivars of Citrus Fruit

2.2.1. Ethane Efflux Method for Calculation of Gas Diffusion Resistance

A modified ethane efflux method comprising an absorption process and an efflux process was performed using five samples for each of the citrus fruit cultivars to calculate the resistance to gas diffusion from the ethane effluence behavior in the effluence process according to the modified method of Dirpan et al. (2016) [11].

During the absorption process, as shown in Figure 1, a single fruit was placed in an acrylic box with two vent holes and a septum hole. The temperature was 20 °C, maintained by an incubator. Subsequently, a constant amount of air containing ca. 35,000 ppm of ethane gas flowed through a mixing box into the box with two vent holes for 4 h to diffuse the ethane gas into the tissue of the fruit.

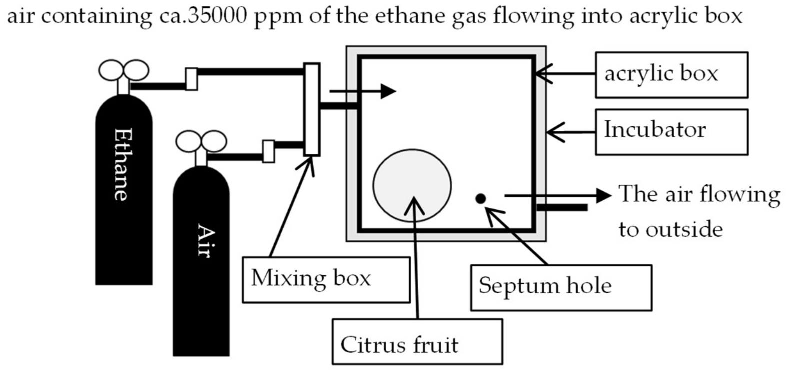


Figure 1. Schematic diagram of an experimental apparatus in the absorption process for measuring gas diffusion resistance.

After 4 h, as shown in Figure 2, the fruit was transferred to an acrylic box with a septum hole for the effluence process, and the box was closed immediately. Subsequently, the internal ethane gas absorbed to the fruit was diffused to the headspace in a closed box at 20 °C, as maintained by incubator. An alternating current fan was placed in the closed box to ensure uniform ethane gas concentration in the headspace.

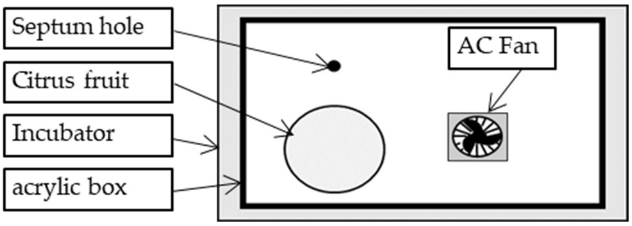


Figure 2. Schematic diagram of an experimental apparatus in the effluence process for measuring gas diffusion resistance.

The gas in each of the boxes for absorption and effluence process was sampled by a syringe passing a needle through a septum, whose role was to increase the gas tightness of the boxes at the time of the gas sampling. The sampling gas of 0.5 mL in the syringe was used to analyze the ethane gas concentration. The ethane gas concentration was analyzed by gas chromatography (model GC-2014; Shimadzu, Kyoto, Japan) coupled with a flame ionization detector (FID) and a 200 × 0.3 cm I.D. stainless steel column containing activated alumina 80/100 mesh. Nitrogen gas was used as the carrier gas for the gas chromatography process. The injector, column, and detector temperatures for gas chromatography were 80, 70, and 90 °C, respectively.

2.2.2. Measuring Construction Characteristics

After the effluence process, the volume, stomatal density, surface area, outer skin thickness, and void ratio of the same fruits were measured. The fruit volume (V) was determined by measuring the buoyant force on the fruit in water. The stomatal density on the outer skin was measured by microscopic observation of the fruit outer skin surface of three samples that were transferred on instant adhesive using Suzuki's universal micro-printing (Sump) method. The surface area of the fruits was measured as follows: The outer skin of the fruit was cut and photographed using a digital camera. The dimensions of the outer skin on the picture used as the surface dimension of the citrus fruit (A) were measured using Image J. Additionally, the thickness of the cut outer skin was measured at three locations on the equatorial plane using a digital caliper. The void ratio of the fruits was calculated from the volume (V), weight (W), and real density (D) of the fruits using Equation (1). Real density (D) was measured using a pycnometer filled with fruits minced by a juicer.

$$void\ ratio\ (\%) = 100 \times \left(1 - \frac{W}{V \times D}\right) \tag{1}$$

Additionally, the void volume in citrus fruit (V_{in}) was calculated as the apparent citrus fruit volume (V) minus the true volume, which was the weight (W) divided by the real density (D), as shown in Equation (2).

$$V_{in} = V - \frac{W}{D} \tag{2}$$

Table 1 shows the weight (W; g), apparent volume (V; cm^3), internal void volume (V_{in}; cm^3), and surface dimension (A; cm^2) of all cultivars of citrus fruits.

Table 1. A weight and an apparent volume and an internal void volume and a surface dimension in all cultivars of the citrus fruits.

Cultivars	Weight	Apparent Volume	Internal Void Volume	Surface Dimension
C. spp "Shiranui"	216.4 ± 21.3 g	211.1 ± 16.8 cm^3	33.5 ± 3.2 cm^3	156.0 ± 15.8 cm^2
C. unshu "Nankan No.20"	160.8 ± 5.0 g	175.7 ± 12.6 cm^3	42.7 ± 10.0 cm^3	141.0 ± 14.4 cm^2
C. spp "Harehime"	172.1 ± 17.2 g	185.4 ± 22.4 cm^3	41.9 ± 10.0 cm^3	171.0 ± 28.4 cm^2
C. iyo "Miyauchi iyokan"	228.8 ± 12.1 g	263.8 ± 8.4 cm^3	72.8 ± 6.3 cm^3	190.3 ± 12.7 cm^2
C. spp "Setoka"	201.8 ± 11.7 g	191.1 ± 12.6 cm^3	23.6 ± 4.0 cm^3	146.9 ± 11.8 cm^2
C. spp "Ehime Kashi No.28"	212.2 ± 17.1 g	199.1 ± 17.5 cm^3	21.5 ± 3.9 cm^3	139.4 ± 11.2 cm^2
C. spp "Kanpei"	217.5 ± 37.0 g	218.4 ± 19.0 cm^3	26.9 ± 7.9 cm^3	171.9 ± 17.8 cm^2

2.2.3. Calculation of Gas Diffusion Resistance

A formula for calculating an external concentration of ethane gas in the effluence process was derived from differential equation of Fick's first law of diffusion, as shown in Equation (3). This solution of a differential equation was conducted on the base of Dirpan and Hikida's method [3,11]:

$$^{ds}/_{dt} = k\left(C_{in}^{t} - C_{out}^{t}\right) \tag{3}$$

where $^{ds}/_{dt}$ and k is the diffusion rate of ethane gas and the ethane gas diffusion kinetic constant. $C_{in}^{t} - C_{out}^{t}$ is ethane gas concentration gradient between the inside and outside of the citrus fruit, where the gradient ($C_{in}^{t} - C_{out}^{t}$) fluctuates according to a time lapse in the effluence process.

The differential equation of Equation (3) was solved, as shown in Equation (4).

$$C_{out}^{t} = C_{out}^{\infty}\left(1 - e^{-kt}\right) \tag{4}$$

where C_{out}^{t} is the ethane gas concentration sampling from the headspace of the closed box of the effluence process at each of the time, which can be calculated from the saturated ethane gas concentration in the headspace (C_{out}^{∞}), time (t), and ethane gas diffusion kinetic constant (k). The saturated ethane gas concentration in the headspace (C_{out}^{∞}) can be calculated by dividing the total amount of ethane gas absorbed into the void space in fruits during the absorption process by the total volume of the headspace in the closed box (V_{out}) and void in citrus fruit (V_{in}), as shown in Equation (5).

$$C_{out}^{\infty} = \frac{C_{in}^{t=0} \cdot V_{in}}{V_{in} + V_{out}} \tag{5}$$

where $C_{in}^{t=0}$ is internal gas concentration in the fruits at beginning of the effluence process, which is equivalent to external gas concentration in the box at end of the absorption process. The total amount of ethane gas absorbed into the fruits until end of the absorption process was calculated by multiplying the ethane gas concentration in the box at end of the absorption process with void in the fruit ($C_{in}^{t=0} \cdot V_{in}$). The void volume in citrus fruit (V_{in}) was used as shown in Section 2.2.2, and the volume of the headspace in the closed box (V_{out}) was used as the total volume of the box inside minus the volume of the alternating current fan and the fruit.

The ethane gas diffusion kinetic constant (k) was calculated from the least-square method, approximating between the calculation result in Equation (4) (C_{in}^{t}) and the experimental result of the ethane gas concentration during the diffusion experiment. The resistance to gas diffusion was calculated using the ethane gas diffusion kinetic constant (k), void volume in citrus fruit (V_{in}), volume of headspace in the closed box (V_{out}), and surface dimension of the citrus fruit (A), as shown in Equation (6).

$$R = \frac{V_{in} + V_{out}}{V_{in} V_{out}} \frac{A}{k} \tag{6}$$

2.3. Measurement of Internal Gas Components Related to the Respiration for Cultivars of Citrus Fruit

Method of extracting internal gases of fruits was performed on the base of Hikida's method [3]. The internal gas component related with respiration was investigated using another five fruits of the citrus cultivars, respectively. After one night of storage at 10 °C, the samples stored at 10 °C or 25 °C for several weeks were used to extract the internal gas of the fruits. A schematic diagram of the vacuum extracting of the internal gas from the fruits is shown in Figure 3. Each of the fruits was placed in a beaker filled with water. To collect the internal gas after vacuum, a funnel filled with water and covered with a silicon stopper was placed above the fruit. The beaker was placed in a desiccator, and the air in the desiccator was vacuumed at −0.08 MPa for 1 min using an air compressor (DA-15D, ULVAC, Inc., Kanagawa, Japan). After the vacuum, the collected gas at the top of the funnels was sampled using a syringe, passing a needle through the silicon stopper.

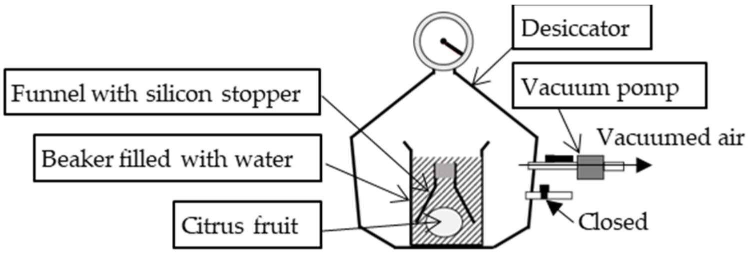


Figure 3. Schematic diagram of an experimental apparatus of extracting internal gas of citrus fruits to measure internal gas concentration.

The sampling gas of 0.5 mL in the syringe was used for the analysis of oxygen and carbon dioxide concentrations. The oxygen and carbon dioxide concentrations were analyzed using a gas chromatograph (model GC-8A; Shimadzu, Kyoto, Japan) coupled with a thermal conductivity detector (TCD) and a 1.8 m × ϕ1/4 O.D. stainless steel column WG-100. Helium gas was used as the carrier gas for gas chromatography. The temperatures of the injector and column used for gas chromatography were 80 °C and 50 °C, respectively.

2.4. Statistical Analysis

All experimental results were expressed as mean ± standard deviation using data of five samples, respectively. Comparing among experimental results, statistical significance was verified by Tukey–Kramer's method ($p < 0.05$).

3. Results and Discussions

3.1. Gas Diffusion Resistance for Seven Citrus Fruit Cultivars

Figure 4 shows the ethane gas concentration in the closed box during the effluence process for *C. spp "Shiranui"* and *"Ehime Kashi No.28"* of citrus fruits; these are example results of minimum and maximum gas resistance cultivars. In all of samples, a rapid effluence of the ethane gas from the fruits was observed at initial period of the process, while the effluence was suppressed secondly. Finally, an equilibrium of the gas concentration was observed in each of samples. On the other hand, the effluence behavior of the ethane gas differed considerably from those of the cultivars. These differences could be a result of the gas resistance of each of the cultivars. Thus, the resistance to gas diffusion between the inside of the fruit and the atmosphere was calculated from the approximation between calculating formula (Equation (4)) and the experimental results of the external ethane gas concentration, as shown in Section 2.2, respectively.

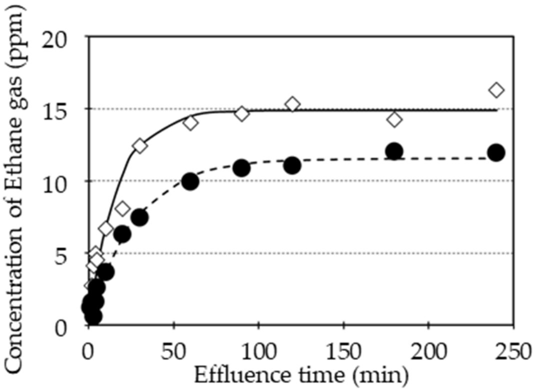


Figure 4. Ethane gas concentration of experimental results in the closed box during the effluence process and approximating results utilizing calculating formula (Equation (4)) for *C. spp "Shiranui"* and *"Ehime Kashi No.28"* of citrus fruits, which are example results of the minimum and maximum gas effusion resistance cultivars (n = 1). The experimental results of ethane gas concentration are indicated for *C. spp "Shiranui"* using "●" and for *C. spp "Ehime Kashi No.28"* using "◇". The approximate results are indicated for *C. spp "Shiranui"* using a "dotted curve line" and for *C. spp "Ehime Kashi No.28"* using "continuous curve line".

Figure 5 shows the resistance to gas diffusion between the inside of the fruit and the atmosphere using a calculation from each result of the ethane gas diffusion experiments for the seven cultivars of citrus fruit. The gas diffusion resistance was 178.8 ± 82.8 s/cm for *Citrus spp "Shiranui"*, 175.1 ± 51.1 s/cm for *C. unshu "Nankan No.20"*, 129.2 ± 27.1 s/cm for *C. spp "Harehime"*, 108.4 ± 45.8 s/cm for *C. iyo "Miyauchi iyokan"*, 100.6 ± 43.2 s/cm for *C. spp "Setoka"*, 52.7 ± 17.2 s/cm for *C. spp "Ehime Kashi No.28"*, and 47.9 ± 5.0 s/cm for *C. spp "Kanpei"*. Although all cultivars of the fruits were grouped into the genus Citrus, each citrus fruit cultivar had a different resistance to gas diffusion. Considering that the constructions of each of the cultivars of citrus fruit were varied, the fruits constructions might affect the gas diffusion resistance of each cultivar. Thus, a comparison was conducted among the gas diffusion resistances and the fruit constructions.

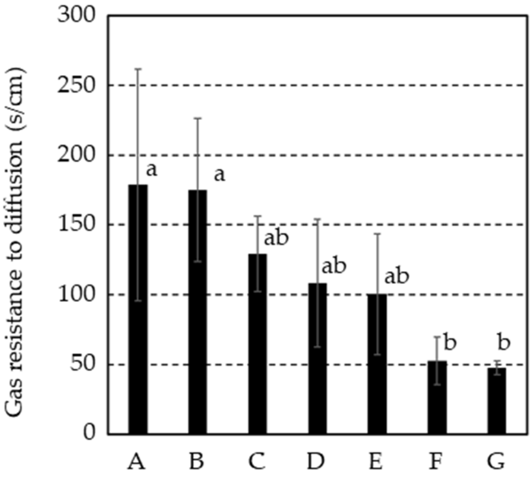
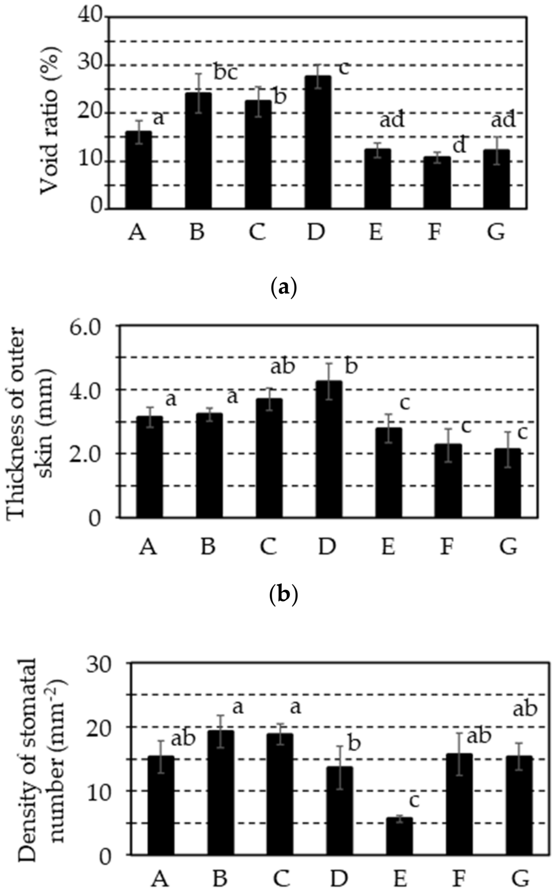


Figure 5. Gas diffusion resistance between the inside of the fruit and the atmosphere utilizing a calculation from each result of the ethane gas diffusion for seven cultivars of citrus fruit. *C. spp "Shiranui"* is indicated with "A"; *C. unshu "Nankan No.20"* is indicated with "B"; *C. spp "Harehime"* is indicated with "C"; *C. iyo "Miyauchi iyokan"* is indicated with "D"; *C. spp "Setoka"* is indicated with "E"; *C. spp "Ehime Kashi No.28"* is indicated with "F"; *C. spp "Kanpei"* is indicated with "G". Results and error bar were shown by mean values and standard deviation of 5 samples (n = 5). Significant difference in the gas diffusion resistance among all cultivars of citrus fruit was evaluated by Tukey–Kramer's method ($p < 0.05$). The different letters indicate a significant difference.

Figure 6 shows the void ratio of the inside of the fruit, the outer skin thickness, and the stomatal number density for each of the seven fruit cultivars. These same fruits were investigated after the gas diffusion resistance experiment. The void of the inside of the fruit is a route for gas diffusion in the inside of the fruit [1,18]. Therefore, the gas diffusion resistance of the inside of the fruit might increase according to a decrease in the void ratio of the inside of the fruit. However, the cultivars of citrus fruits with lower gas diffusion resistance, such as *C. spp "Ehime Kashi No.28"* and *C. spp "Kanpei"*, had a low ratio of void for the inside of the fruit, as shown in Figure 6a. From this result, it was speculated that the void ratio in the fruits has no considerable effect on the gas diffusion resistance between the inside of the fruit and the atmosphere, which was similarly reported for Japanese pear cultivars [12].



(**a**)

(**b**)

(**c**)

Figure 6. Comparison of construction for seven cultivars of citrus fruit. The construction of citrus fruits for (**a**) void ratio, (**b**) thickness of outer skin, and (**c**) density of stomatal number was investigated utilizing the same sample for measuring gas diffusion resistance. *C. spp "Shiranui"* is indicated using "A"; *C. unshu "Nankan No.20"* is indicated using "B"; *C. spp "Harehime"* is indicated using "C"; *C. iyo "Miyauchi iyokan"* is indicated using "D"; *C. spp "Setoka"* is indicated using "E"; *C. spp "Ehime Kashi No.28"* is indicated using "F"; *C. spp "Kanpei"* is indicated using "G". Results and error bar were shown by mean values and standard deviation of 5 samples (n = 5). Significant difference in the gas diffusion resistance among all cultivars of citrus fruit was evaluated by Tukey–Kramer's method ($p < 0.05$). The different letters indicate a significant difference.

The cultivars of the citrus fruits with lower gas diffusion resistance, such as *C. spp "Ehime Kashi No.28"* and *C. spp "Kanpei"*, had a low outer skin thickness in comparison with the other cultivars, as shown in Figure 6b. The outer skin of horticultural produce is the outermost construction separating the inside of the fruit from the atmosphere; it plays a major role in the barrier to gas exchange [1,18]. Schotsmans et al. (2004) [18] reported that the apple cultivar *"Jonica"*, with a thin outer skin thickness (20 µm), had a higher gas diffusion resistance than *"Braeburn"*, with a low outer skin thickness (14 µm) [15]. Therefore, differences in outer skin thickness between 2.1 and 4.2 mm for citrus fruits could have a considerable effect on the gas diffusion resistance for each cultivar.

However, the citrus fruits of *C. spp "Setoka"*, with an outer skin thickness equivalent to that of *C. spp "Ehime Kashi No.28"* and *C. spp "Kanpei"*, had a higher gas diffusion resistance than these cultivars. Focusing on the density of the stomatal number on the outer skin surface, that for the citrus fruits of *C. spp "Setoka"* was less than that for fruits of the other cultivars; the significance of this is shown in Figure 6c ($p < 0.05$). Since a stoma is a route for gas diffusion between the inside of the fruit and the atmosphere [1,18], the stomatal number density could affect the gas diffusion resistance. Therefore, although the citrus fruits of *C. spp "Setoka"* had a low outer skin thickness, the high gas diffusion resistance of the citrus fruits of *C. spp "Setoka"* might be induced by the low stomatal number density compared to those of *C. spp "Ehime Kashi No.28"* and *C. spp "Kanpei"*.

From these results, resistance to gas diffusion between the inside of the fruit and the atmosphere could be generated from multiple constructions of citrus fruits, such as outer skin thickness and stomatal number density.

3.2. Fluctuation in Internal Gas Concentration in the Fruits of Citrus Unshu "Nankan No.20" During Storage

Figure 7 shows the gas concentration of O_2 and CO_2 in the fruits of *C. unshu "Nankan No.20"* stored at 10 °C or 25 °C for 4 weeks. The atmospheric gas concentration in the laboratory room was controlled at 0.3 ± 0.0% for CO_2 and 21.2 ± 1.6% for O_2; meanwhile, in the fruits, even before storage, the gas concentration was 2.0 ± 0.2% for CO_2 and 21.0 ± 0.2% for O_2. Therefore, the CO_2 gas concentrations inside the fruits might be constantly higher than those in the atmosphere. When the fruits were stored at 10 °C for 4 weeks, the gas concentrations were 1.8 ± 0.4% for CO_2 and 20.5 ± 0.2% for O_2, and a slight fluctuation in the internal gas concentrations was observed during storage. However, at 25 °C, the fluctuation in the gas concentration during the storage time was considerable. After a storage time of 4 weeks, the CO_2 gas concentration increased to 6.2 ± 0.8% and O_2 decreased to 16.0 ± 1.1%. A fruit constantly undergoes respiration, and its respiration results in O_2 consumption and CO_2 synthesis in the fruits. Normally, gases transport between the atmosphere and the inside of the fruit for a supply of O_2 gas and an effusion of CO_2 gas, conducted by passing through the outer skin and other constructive elements. When the gas concentration fluctuation induced by respiration of fruits is not considerable, gas exchange can result in the maintenance of gas concentrations during storage at certain temperatures, e.g., at 10 °C. However, if respiration is activated by the high storage temperature, the supply and effusion of the gases can be insufficiently conducted, which can result in a decrease in O_2 and an increase in CO_2 of the internal gas concentration. The internal gas concentration of fruit can be determined by the balance between its respiration and gas exchange [1]. As a result of the balance, comparing with the gas concentration in the fruits before storage, storage at 10 °C maintained the gas concentration, while storage at 25 °C induced a decrease in O_2 and an increase in CO_2. Additionally, in observations of visible features, it seemed that the amount of gas extracted by the vacuum from the fruit stored at 10 °C was constant regardless of the storage period, while that at 25 °C decreased with the extension of the storage period; this is a similar observation to the report that the internal gas amount of the onion bulb decreased with an increase in storage temperature [17]. Therefore, as observed at 25 °C, active respiration could cause not only fluctuations of the gas concentration but also a decrease in the gaseous amount in the fruit.

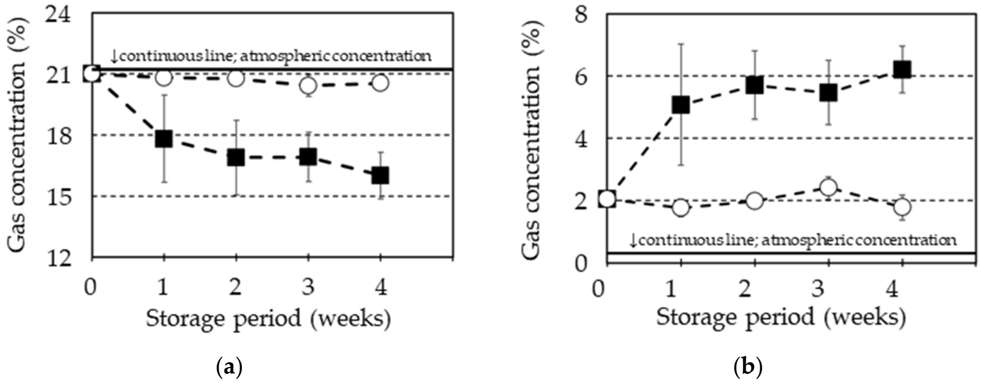


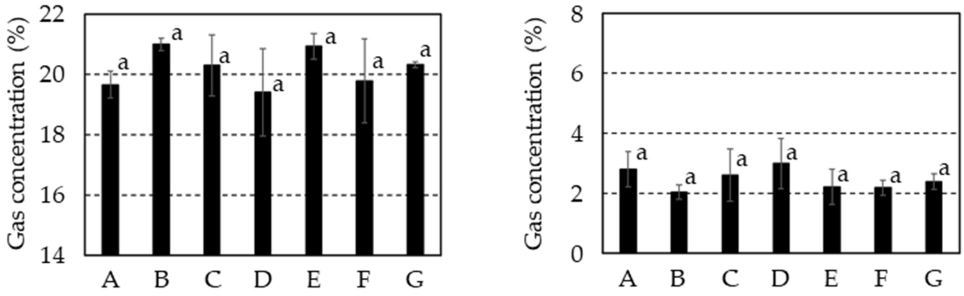
Figure 7. Fluctuation in internal gas concentration for the fruits of *C. unshu "Nankan No.20"* during storage at 10 °C or 25 °C for 4 weeks. Each gas concentration was investigated for (**a**) O_2 and (**b**) CO_2. Atmospheric gas concentration is indicated as a "continuous line"; storage at 10 °C is indicated with "○"; storage at 25 °C is indicated with "■". Results and error bar were shown by mean values and standard deviation of 5 samples (n = 5).

3.3. Comparison of Internal Gas Concentration Among Seven Cultivars of Citrus Fruits

In the fruits of *C. unshu "Nankan No.20"*, considerable fluctuation in the gas concentration was not observed after storage at 25 °C for longer than 2 weeks. Thus, after 2 weeks of storage, the citrus fruits were utilized to compare the internal gas concentrations among the cultivars.

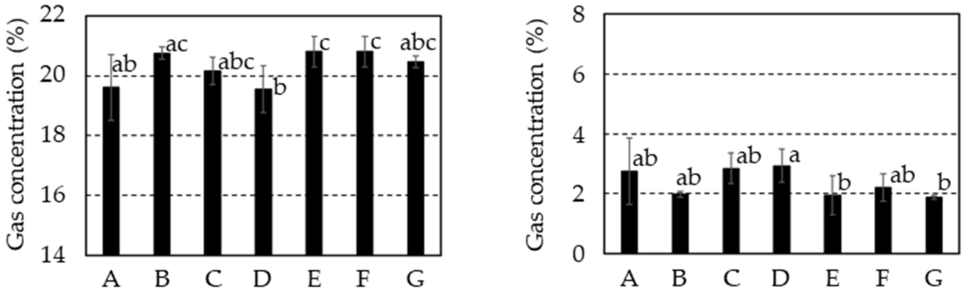
Figures 8–10 show the internal gas concentrations of the fruits which were utilized before storage and after storage at 10 °C and 25 °C for 2 weeks. In the fruits for all of the cultivars before the storage, as shown in Figure 8, there were gas concentrations of 2.0–3.0% for CO_2 and 19.4–20.1% for O_2. In the fruits stored at 10 °C, there were gas concentrations of 2.0–2.9% for CO_2 and 19.5–20.8% for O_2, as shown in Figure 9. Comparing the internal gas concentrations of the fruits before and after storage at 10 °C, in all of the cultivars, no considerable effect of storage on the internal gas concentrations of the fruits was observed. After storage at 25 °C, as shown in Figure 10, an increase in CO_2 concentration and a decrease in O_2 concentration were observed compared to the pre-stored fruits of all of the cultivars. Therefore, aside from the fruits of *C. unshu "Nankan No.20"*, fluctuations in the internal gas concentrations could be affected by active respiration at 25 °C in all the cultivars. Additionally, focusing on the difference in the internal gas concentration among the cultivars, a significant difference was observed in the fruits stored at 25 °C ($p < 0.05$), in contrast with comparing with the pre-stored fruits and the fruits stored at 10 °C. With the increase in the gas diffusion resistance of the cultivars fruit, the CO_2 concentration tended to increase and the O_2 concentration tended to decrease after storage at 25 °C. When active respiration induced fluctuation in the internal gas concentration, the high gas diffusion resistance of the fruits could limit the gas exchange between the atmosphere and the inside of the fruits, which could maintain fluctuated internal gas concentration [10]. Therefore, one of the factors resulting in an increase in CO_2 concentration and a decrease in O_2 concentration after storage at 25 °C may be the high gas diffusion resistance of the fruits.

Additional experiments were performed to investigate the role of the outer skin in the internal gas of citrus fruits since outer skin of thickness and stomatal number density in the citrus fruits affected the gas diffusion resistance considerably. In additional experiments, the fruits of *C. unshu "Nankan No.20"* after storage at 25 °C for 2 weeks were utilized to investigate the approximate internal gas volume and the gas concentration in the fruits before and after peeling the outer skin flavedo, as shown in Table 2. In visible observation, it seemed that the amount of extracted gas from the unpeeled fruit was approximately 20–30 mL, while that from the peeled fruits was approximately 3–5 mL. Since the amount of the extracted gas was increased by the presence of the outer skin, the outer skin could play an important role in holding gases in the fruit. Additionally, the flesh part of the peeled fruits, called segments and vesicles, could contain a small amount of gas compared to the whole fruit.



(**a**) (**b**)

Figure 8. Internal gas concentration for seven cultivars of citrus fruit before storage. Each gas concentration was investigated for (**a**) O_2 and (**b**) CO_2. *C. spp "Shiranui"* is indicated using "A"; *C. unshu "Nankan No.20"* is indicated using "B"; *C. spp "Harehime"* is indicated using "C"; *C. iyo "Miyauchi iyokan"* is indicated using "D"; *C. spp "Setoka"* is indicated using "E"; *C. spp "Ehime Kashi No.28"* is indicated using "F"; *C. spp "Kanpei"* is indicated using "G". Results and error bar were shown by mean values and standard deviation of 5 samples (n = 5). Significant difference in the gas diffusion resistance among all breeds of citrus fruit was evaluated by Tukey–Kramer's method ($p < 0.05$). The different letters indicate a significant difference.

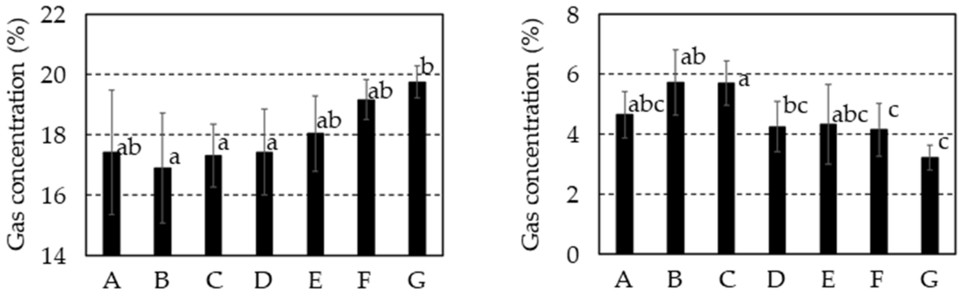


(**a**) (**b**)

Figure 9. Internal gas concentration for seven cultivars of citrus fruit after storage at 10 °C for 2 weeks. Each gas concentration was investigated for (**a**) O_2 and (**b**) CO_2. *C. spp "Shiranui"* is indicated using "A"; *C. unshu "Nankan No.20"* is indicated using "B"; *C. spp "Harehime"* is indicated using "C"; *C. iyo "Miyauchi iyokan"* is indicated using "D"; *C. spp "Setoka"* is indicated using "E"; *C. spp "Ehime Kashi No.28"* is indicated using "F"; *C. spp "Kanpei"* is indicated using "G". Results and error bar were shown by mean values and standard deviation of 5 samples (n = 5). Significant difference in the gas diffusion resistance among all breeds of citrus fruit was evaluated by Tukey–Kramer's method ($p < 0.05$). The different letters indicate a significant difference.

Table 2. The approximate internal gas volume and the gas concentration in the fruits before and after peeling the outer skin flavedo for *C. unshu "Nankan No.20"*.

	Internal Gas Volume in Visible Observation	CO_2 Concentration	O_2 Concentration
Unpeeled fruit	20–30 mL	4.1 ± 0.6%	18.7 ± 0.8%
Peeled fruit	3–5 mL	6.0 ± 1.0%	18.0 ± 1.9%



(**a**)

(**b**)

Figure 10. Internal gas concentration for seven cultivars of citrus fruit after storage at 25 °C for 2 weeks. Each gas concentration was investigated for (**a**) O_2 and (**b**) CO_2. *C. spp "Shiranui"* is indicated using "A"; *C. unshu "Nankan No.20"* is indicated using "B"; *C. spp "Harehime"* is indicated using "C"; *C. iyo "Miyauchi iyokan"* is indicated using "D"; *C. spp "Setoka"* is indicated using "E"; *C. spp "Ehime Kashi No.28"* is indicated using "F"; *C. spp "Kanpei"* is indicated using "G". Results and error bar were shown by mean values and standard deviation of 5 samples (n = 5). Significant difference in the gas diffusion resistance among all breeds of citrus fruit was evaluated by Tukey–Kramer's method ($p < 0.05$). The different letters indicate a significant difference.

In the unpeeled fruits, it was observed that CO_2 and O_2 of the gas concentrations were 4.1 ± 0.6% and 18.7 ± 0.8%. In the peeled fruits, it was observed that CO_2 and O_2 of the gas concentrations were 6.0 ± 1.0% and 18.0 ± 1.9%. Although no difference in O_2 concentration was observed, the CO_2 concentration was higher in the unpeeled fruits than in the peeled fruits. Therefore, CO_2 concentration could increase according to the depth of the inside of the fruit, although it was unclear why the effect of fruit depth on O_2 concentration was not observed.

4. Conclusions

In this study, the gas diffusion resistance and internal gas concentration of seven citrus fruit cultivars were investigated. The results showed that the gas diffusion resistance of the seven cultivars varied according to the characteristics of fruit construction, such as the outer skin thickness and the stomatal number density. Although the internal gas concentration of the fruits involved high CO_2 concentration and low O_2 concentration before storage, storage at 25 °C promoted an increase in CO_2 concentration and a decrease in O_2 concentration. Additionally, the CO_2 concentration tended to increase and the O_2 concentration tended to decrease with an increase in the gas diffusion resistance of fruit cultivars. In addition, the outer skin of citrus fruit may play an important role in holding internal gases.

From these results, it was clarified that the internal gas concentration varied according to the gas diffusion resistance for each of the citrus fruits, which affected the respiration of citrus fruits. Considering that maintaining a level of high CO_2 concentration and low O_2 concentration in the fruits was observed even the fruits with a minimum gas diffusion resistance such as *C. spp "Kanpei"*, citrus fruit may have a system that gas diffusion resistance self-controls internal gas concentration to suppress their respiration. Although this study clarified a clear relationship between the gas diffusion resistance and the construction characteristics of citrus fruits, the resistance of citrus fruit can be related to the outer skin thickness and stomatal number density. Therefore, if the construction characteristics of citrus fruits are modified by genome editing or bleeding, a new citrus fruit cultivar that is excellent in terms of self-controlling respiration may be created. To create this new citrus cultivar, further studies focusing on the relationship between the gas diffusion resistance and construction characteristics of citrus fruits are required.

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