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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：　Resistance to Gas Diffusion in Internal Tissues of Citrus Fruit
Title of Dissertation　(カンキツ果実の組織内ガス拡散抵抗)

学位論文要約：
Dissertation Summary

Citrus is the most widely produced fruit. It has many varieties and it grows in more than 80 countries. Considering the therapeutic value of these fruits and human health awareness, citrus gets world's interest. Hence, the consumption degree of this fruit tends to increase. Japan is a major citrus fruit-producing country, and Ehime Prefecture is one of the main citrus fruit producing regions in Japan. As many as 20 major citrus varieties are cultivated in Ehime. Citrus fruit production concerns for the sustainability challenges, including pesticide use, postharvest quality, and change of consumer preferences. In this research, we focus on the postharvest aspect.

A time span between harvest and consumption is critical period for horticultural crops because they potentially lose the quantity and quality during this time. In fresh fruits and vegetables, the degree of the losing is significant, depending on the following conditions such as the commodity, cultivar, and handling method. While in developed countries, the potential lose range from 5 to 25%, in developing countries range from 20 to 50%. To reduce these losses, it is suggested that understanding the biological and environmental factors involved in deterioration should be firstly considered before choosing proper handling methods and postharvest technologies that delay senescence and maintain the best possible quality. Further, because fresh horticultural crops are diverse in morphological structure, in composition, and in general physiology, commodity requirements and recommendations for maximum postharvest life vary among the commodities.

There are some handling methods and postharvest technologies can be used to maintain the quality of agricultural product, such as: pre-cooling, curing, waxing or coating, chemical treatment, heat treatment, modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage. Both MAP and CA storage have been applied in the food industry for about 90 years to extend shelf life and maintain quality of fresh and fresh-cut foods. Recently, MAP has experienced a rapid development in both scientific and industrial communities, which was one of the most appropriate and practical technologies for packaging fresh and fresh-cut produce. Also CA storage has recently used for storing fruits and vegetables in form of bulk storage where natural gaseous environment is intentionally altered, and is precisely maintained at this altered atmosphere throughout the distribution or storage period, regardless of any environmental variations.

Creating an optimum gas concentration around fruit and vegetables is the purpose of using modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage. However, the external atmosphere of fresh products in MAP and CA storage differs from the internal atmosphere. Therefore, we can expect to improve MAP and CA storage techniques by establishing a prediction and control method for internal atmosphere. Because the internal gas concentration for O_2 and CO_2 were shown to be predicted by the knowledge of the gas exchange rate and resistance to gas diffusion in fresh products when the storage gas condition is known, knowledge of resistance is essential. Therefore, the aim of this research is to determine the intercellular space volume (V_{in}) of citrus fruits and to develop a mathematical modelling to predict V_{in}. Then, this model was applied to examine various citrus size on the resistance to gas diffusion in citrus fruit. Lastly, the aim of the research is to propose an improved method for measuring the resistance of citrus Iyo fruits (*Citrus iyo hort. Ex Tanaka*) to gas diffusion and to calculate the effect of temperature and fruit size on resistance, the conversion to resistances for O_2 and CO_2 gases and the average value and variation in resistance.

(Chapter 1) According to a number of gas exchange studies, V_{in} of fruit is an important factor to be known. Some studies have shown that gas exchange and respiration in fruit are directly influenced by V_{in}, and fruit tissue with a small V_{in} results in low respiration. Other studies focusing on V_{in} in apples and pears have shown that fruit with greater V_{in} or porosity, that is, the ratio of V_{in} and total volume of a fruit, had higher internal gas diffusion and became softer or more mealy. Furthermore, a study on tolerance to controlled atmosphere (CA) storage has suggested that this tolerance depends on V_{in} or porosity. For instance, although the apple cultivar Cox's Orange is characterized by low porosity and has a low tolerance to CA storage, fruit with high porosity tends to have a high tolerance to CA storage.

From the above studies, it can be concluded that V_{in} is an important factor in estimating sensitivity of fruit to low levels of O_2, high levels of CO_2, or both in the surrounding atmosphere and in determining appropriate concentrations for modified atmosphere packaging (MAP) and CA storage. Moreover, to develop MAP or CA storage techniques focusing on citrus fruit, gas diffusion characteristics in plant organs and respiration function in the intercellular space are important to be studied. These knowledge can be used to establish a prediction and control method of O_2 and CO_2 concentrations in the intercellular space. In this case, the V_{in} value of citrus fruit is required. Therefore, the objective of this study is to determine V_{in} using the pycnometric method and to develop a simple prediction method for V_{in}. The pycnometric method is time consuming because ground samples are required. Accordingly, a prediction method can be simply applied to determine the V_{in} of citrus fruits.

The experiment used eight species of freshly harvested citrus fruit at Ehime University farm: Iyokan (*Citrus iyo Hort. ex Tanaka*), Navel (*Citrus sinensis* L. Osbeck var. *brasiliensis* Tanaka), Ponkan (*Citrus reticulata* Blanco), Siranui (Citrus unshiu Marcov x Citrus sinensis Osbeck x *Citrus reticulata* Blanco), Amanatsu (*Citrus natsudaidai* Hayata), 'Kiyomi' tangor (*Citrus unshiu* Marcov. forma *miyagawa-wase* x *C. sinensis* Osbeck), Lime (*Citrus aurantifolia*), and Unshu (*Citrus unshiu* Marc. cv. Kuno). Each species consisted of five sizes (S, M, L, 2L, and 3L), except Lime, for which only two sizes were used (small and large) because of the absence of size standards. The citrus fruits were measured just after harvest.

In determining V_{in}, the mass (m), vertical and horizontal diameters (vd, hd), total volume (V_t), and real density (P_t) of eight species of citrus fruits of different sizes were measured. Real density (P_t) of citrus tissue was measured using the pycnometric technique. A technique based on Archimedes' principle was used to determine the total volume (V_t) of fruit. Buoyancy was measured using an electric balance accurate to 0.1 g when a sample was submerged in water. Sphere equivalent volume (V_s) was also calculated by assuming that the fruit is a sphere with a diameter the average of vd, hd1, and hd2.

The results showed that each species had various V_{in}, which depended on fruit size; in other words, the larger the size of the fruit, the larger the Vin. However, the levels of porosity (Ø) in each species were not fully proportional to fruit size. For all species, V_{in} ranged from 4.2 cm^3 to 142.2 cm^3, in which 3L Amanatsu was the highest. The porosity of all species ranged from 6.7% to 29.4% which Amanatsu and Lime had a porosity proportional to their size, the remaining samples showed otherwise.

The mean apparent density of the citrus segments (1.0147 g/cm^3) was greater than that of intact citruses (0.8901 g/cm^3), indicating that the porosity of intact fruit is greater than that of a segment. The porosity of citrus segments was only 2.6% while the porosity of whole fruit was 15.2%, suggesting that the majority of intercellular space is outside the segments. Most V_{in} is in the central cavity and under the skin of the citrus.

Total real densities ranged from a minimum of 1.0409 g/cm^3 to a maximum of 1.0838 g/cm^3 with a very small standard deviation of 0.0077 g/cm^3 and standard error of 0.00057 g/cm^3, and the total mean was 1.0647 g/cm^3. All the species were therefore considered to have generally the same value as the total mean. On the basis of this mean value, we developed two mathematical models to predict V_{in}. The best model was selected based on the higher values for the determination coefficient (R^2), modeling efficiency (EF), index of agreement (d), and paired *t*-test. R^2 provides a measure of how well the model replicates the measured values. To obtain an acceptable goodness of fit, the values for R^2 must approach 1. EF and d are dimensionless statistics ranging from $-\infty$ to 1 and from 0 to 1, respectively, which suggests that the value should be equal to or greater than 0 for EF and 0.75 for d to evaluate a model precisely.

In the first model, mass (m), total volume (V_t) determined using Archimedes' principle, and real density (P_t) to develop a simple prediction method for V_{in}. By assuming that the values for real density (P_t) are similar in all species based on our findings in this study (1.0647 g/cm^3). The model used to develop the simple prediction method for V_{in} ($V_{in} = V_t - \frac{m}{1.0647}$, where V_{in} and V_t are in cubic centimeters and m is in grams) exhibited a high determination coefficient (R^2 = 0.999), modeling efficiency (EF = 0.999), index of agreement (d = 0.999), and paired t-test value (0.365). It was concluded that the model is suitable for predicting V_{in} of citrus fruits.

In the second model, total volume (V_t) was replaced with sphere equivalent volume (V_s), $V_{in} = V_s - \frac{m}{1.0647}$, where V_{in} and V_s are in cubic centimeters and m is in grams. To validate the model, the measured and predicted values for V_{in} were compared using R^2, EF, d, and the paired *t*-test. Total measured and predicted values from 185 samples validated the model. The values for R^2 (0.860), EF (0.679), and d (0.904) could be generally acceptable. However, the paired *t*-test (0.000) shows that measured and predicted V_{in} were significantly different. The paired *t*-test values of each species were less than 0.05, except for Siranui (0.391), indicating that this model is not suitable for precisely predicting V_{in}.

(Chapter 2) According to the previous chapter, the model for predicting intercellular space volume (V_{in}) is acceptable. Therefore, in this chapter this model is applied to measure resistance to gas diffusion in various citrus sizes.

The knowledge of resistance in gas transport is essential for calculating the internal concentration of O_2 and CO_2 in the fruit when the information of storage gas concentrations are available. Therefore, the understanding of resistance in the MAP and CA storage is needed to predict permissible minimum gas levels in the MAP and CA storage. It will also provide invaluable information of the physiological defect that may develop during the MAP and CA storage time. In Japan, one citrus species sometimes has different sizes when ready to be consumed.

Then it is necessary to preserve their long life based on their sizes with such postharvest technology as CA storage. From the above explanation, it can be concluded that resistance in different sizes is an important factor to be investigated. However, information about the effect of fruit size on resistance is unavailable. Thus, the current research was initiated to study the effect of citrus size in three different sizes of citrus on resistance to gas diffusion.

Citrus Iyo fruit used for measurement were obtained from the Ehime University farm on 27 January 2014 and stored in polyethylene film bags at 5ºC before the experiment.

Fruit of size M (7.3~8.0 cm diameter), L (8.0~8.8 cm) and 3L (9.5~10.2 cm) obtained on 27 January 2014 were used for measurement. Resistance to gas diffusion of the fruit was determined using the ethane efflux method of CAMERON and YANG (1982) with some modification. A single fruit was placed in an acrylic closed box (13 cm in diameter × 15cm in height, 2×10^{-3} m^3) with a constant flow of air containing 1,800 ~ 2,000 ppm ethane for five hours (Fig. 1, step A). This length of time was selected from the preliminary experiments conducted over three, five and seven hours. The ethane concentration in the box was measured with a gas chromatograph (model GC-2014, FID; Shimadzu, Japan) every hour and the total mean of the concentrations was calculated (C_{in}^{0}). The gas chromatograph was coupled with a FID and a 200 cm x 0.3 cm I.D. stainless steel column containing Activated Alumina 80/100 mesh. The temperatures of the injector, column and detector were 80ºC, 70ºC, and 90ºC respectively. Nitrogen was used as the carrier gas. Next, the fruit was transferred quickly to a second acrylic box (29.5 × 29.5 × 12.0 cm, 1.0443×10^{-2} m^3, ethane free) which was quickly closed at time zero (Fig. 1, step B). At the time of sealing, a small AC fan (2.0×10^{-5} m^3) attached to the box was turned on to ensure rapid mixing. To determine the concentration of ethane, 0.5 ml gas samples were withdrawn and measured using the gas chromatograph every five minutes for one hour and every ten minutes for a further hour (total of two hours) through a septum (C^{t}_{out}). After 18 samples were taken, the fan was turned off and adequate time (24 hours) was allowed. The concentration of ethane in the box was then remeasured (C_{out}^{∞}) by with the gas chromatograph.

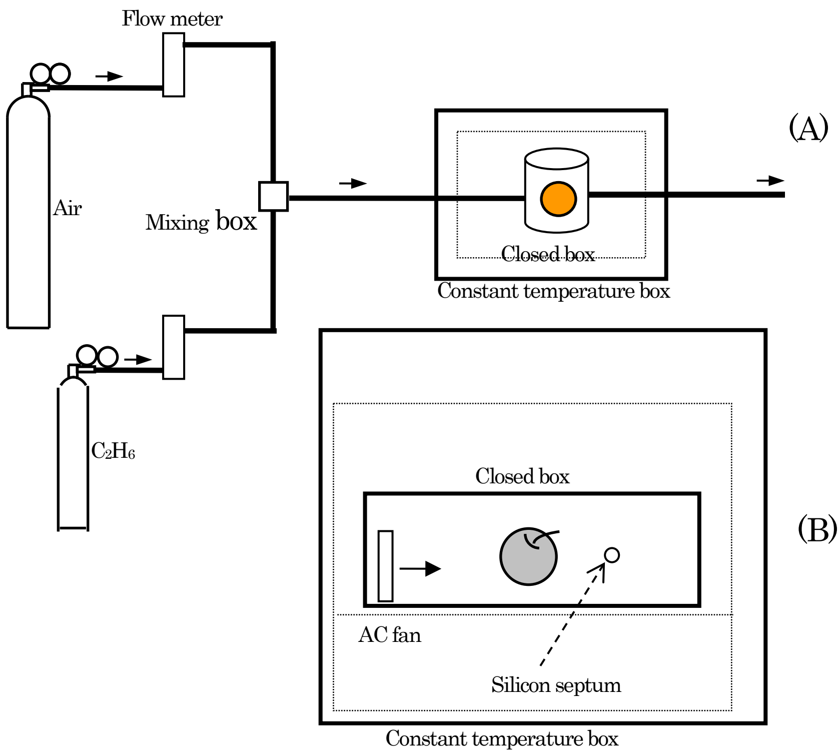

Fig.1 Schematic diagram of the experimental apparatus

Resistance (R) can be measured by the result of first order differential equation for C_{out}^{t} derived from Equation (1). Then, resistance can be measured by using equation (2)

$$\frac{ds}{dt} = \left(C_{in}^{\ t} - C_{out}^{\ t}\right)\frac{A}{R} \tag{1}$$

$$R = \frac{A}{kV_{in}} \tag{2}$$

surface area (A) was calculated from the citrus surface, assuming the citrus to be a perfect sphere. According to previous result in chapter 1, intercellular space volume (V_{in}) can be calculated using Equation (3)

$$V_{in} = V_t - \frac{m}{1.0647} \tag{3}$$

where the total volume (V_t) of fruit was determined by a technique based on Archimedes' principle. Buoyancy was measured using an electric balance accurate to 0.1 g when a sample was submerged in water. Fruit mass (m) was measured using an electric balance accurate to 0.1 g. The accuracy of equation (3) has been investigated before using it for estimating V_{in} of citrus fruit (Chapter 1). The coefficient of determination (R^2) was found to be 0.999. The measurements of resistance based on fruit sizes were performed at 5^0C for each size: M, L, and 3L respectively. All experiments were done in three replications.

The result shows that the highest resistance occurs in 3L size followed by L and M size with 6.84×10^5 s/m, 4.99×10^5 s/m, and 4.33×10^5 s/m respectively. Moreover, Tukey's test revealed differences in the resistance values in different size. This suggests that resistance value significantly differs from the three fruit sizes ($P < 0.01$). Size differences in resistance could be due to anatomical differences such as differences in size of intercellular spaces near the fruit surface; size, number, and distribution of functional lenticels on the fruit surface and thickness and nature of wax deposits of the cuticle.

After obtaining resistance values for ethane in citrus fruits, it is important to calculate the resistance values for CO_2 and O_2. Using Graham's Law, predictions on the relationship between the rate of diffusion and resistance can be made. Therefore, measuring the resistance value for ethane of the citrus and knowing molecular weight of gases permits us to predict the resistance values for CO_2 and O_2 respectively by using Equation (4).

$$\frac{R_2}{R_1} = \sqrt{\frac{M_2}{M_1}} \tag{4}$$

where M_1,M_2= Molecular weights of gases (g/mol), R_1, R_2 = Resistance of gases (s/m).

As a result, comparison of the mean resistance C_2H_6, O_2 and CO_2 indicated that resistance CO_2 and O_2 were higher than resistance C_2H_6. The difference could be due to differences in their molecular weight (30.02, 31.99 and 44.00 for C_2H_6, O_2 and CO_2 respectively) which would affect their relative diffusity.

(Chapter 3) Some studies on measuring resistance have been conducted (BANKS (1985) for apple and potato, KNEE (1991) and PEPPELENBOS and JEKSRUD (1996) for apple), essentially based on CAMERON and YANG (1982). However, this ethane efflux method which measure the ethane concentration flowed out from a sample in a closed box is time consuming, because equilibrium concentration must be measured. For further development of MAP or CA storage techniques focusing on citrus fruit, we measured the resistance of citrus Iyo fruit (*Citrus iyo hort. Ex Tanaka*); one of the citrus varieties which generally stored after harvest by modifying the ethane efflux method of CAMERON and YANG (1982). Therefore, in this study, we propose an improved method of measuring resistance that can shorten the time required to measure, and present the effect of temperature and fruit size on resistance, the conversion to resistances for O_2 and CO_2 gases and the average value and variation in resistance. Citrus Iyo fruit, the size of which falls in the middle class of twenty major citrus varieties cultivated in Ehime Prefecture, is a late-season variety. It is generally harvested in December, stored on farms for about three months and is then distributed until March.

Citrus Iyo fruit of size M (7.3~8.0 cm diameter), L (8.0~8.8 cm) and 3L (9.5~10.2 cm) were used to measure resistance. For sample surface area, size M, L, 2L (8.8~9.5 cm) and 3L were used. Samples used for measurement were obtained from the Ehime University farm on 28 December 2013 and 27 January 2014 and stored in polyethylene film bags at 5ºC before the experiment. Procedure of measuring resistance has been explained in chapter 2. According to CAMERON and YANG (1982), resistance is determined with the following equation:

$$R = \frac{A}{kV_{in}} \tag{2}$$

$$V_{in} = \frac{V_{out}}{C_{in}^{0}} \times C_{out}^{\infty} \tag{5}$$

R : Resistance (s/m)
A : Surface area of a fruit (m^2)
k : First order efflux rate constant (1/s)
V_{in} : Intercellular space volume of a fruit (m^3)
V_{out} : Free volume of closed box (m^3)
C_{in}^{0} : Internal concentration in a fruit at time zero (ppm)
C_{out}^{∞} : Equilibrium concentration in closed box (ppm)

where k was determined as the negative slope of a plot of ln $(1-C^{t}_{out}/C_{out}^{\infty})$ versus time. However, this method is time consuming, because C_{out}^{∞} must be measured. V_{in} in citrus fruit can be determined using our previous model equation in Chapter 1 (Dirpan *et al.*, 2015) :

$$V_{in} = V_t - \frac{m}{1.0647} \tag{3}$$

V_{in} : Intercellular space volume of a fruit (cm^3)
V_t : Total volume of a fruit (cm^3)
m : Mass (g)

In Equation (5), C_{out}^{∞} is obtained with the next equation and k can be determined as the negative slope of a plot of ln $(1-C^{t}_{out}/C_{out}^{\infty})$ versus time:

$$C_{out}^{\infty} = \frac{C_{in}^{0}}{V_{in} + V_{out}} \times V_{in} \tag{6}$$

where V_{out} in Equation (5) was replaced by $(V_{in} + V_{out})$ for precise calculation. Therefore, we can calculate resistance by using Equations (2), (3) and (6) without having to conduct the time consuming experiment. This proposed method was compared with the method of CAMERON and YANG (1982) by using each of the twelve data sets measured at 5ºC and 20ºC for the size M, L, and 3L samples. The mass of the samples was measured using an electric balance accurate to 0.1 g. Total volume of the samples was measured using Archimedes' principle. Ten samples of each size (M, L, 2L and 3L) were used for the measurement. The peel of each sample was carefully removed by knife and the projected area of the peel by a copy machine was measured. The mass and surface area was regressed using the following equation:

$$A=4.2058m^{0.7238},\ R^2=0.97 \tag{7}$$

A : Surface area (cm^2)
m : Mass (g)

Then Equation (7) was used to calculate the surface area of the samples used for the measurement of resistance by substituting the mass. The porosity of the samples for the measurement of resistance was calculated as the ratio of intercellular space volume, which can be determined with Equation (3), to total volume. Skin thickness, consisting of flavedo and albedo, was measured at four locations on the equatorial plane using a digital caliper. Five fruit each of sizes M, L and 3L were used for the measurement.

The proposed method of calculating resistance using Equations (2), (3) and (6) was compared with the method of CAMERON and YANG (1982) by using a paired t-test applied to each of twelve data sets measured at 5 and 20ºC for sample sizes M, L, and 3L. The obtained paired t-test values of 0.776 at 5ºC and 0.887 at 20ºC showed the resistance values measured by the two methods were not statistically different at the 5% level ($P<0.05$). The means of standard error of these data sets (1.13×103 s/m at 5ºC and 1.29×103 s/m at 20ºC) stayed between the upper and lower levels of 95% confidence. It could be stated that there is close agreement between the two methods and the resistance of porous citrus fruit like citrus Iyo can be measured using the proposed method.

Effect of temperature on resistance shows the resistance values of the size L sample at 5, 10 and 20ºC. EBRAHIM *et al*. (2014) showed that the skin resistance values of Japanese pear decreased with increasing temperature. Similar results were obtained in this study. It could be explained that gas diffusivity in the diffusion route from intercellular space to stomata or lenticels of fruit, gas phase diffusion, depends on temperature and increases in temperature. The difference in resistance values at 5 and 20ºC was significant at the 5% level ($P<0.05$) as determined using Tukey's test. The average resistance values were 5.03×10^5, 4.34×10^5 and 3.50×10^5 s/m at 5, 10 and 20ºC, respectively. The average values correlated linearly with temperature ($R^2=0.98$) and decreased about 10^5 s/m with a 10ºC increase in temperature.

Effect of fruit size on resistance shows that the resistance value of ethane for three different sizes at 5 and 20ºC is similar pattern, with the highest value for size 3L followed by L and M. The difference in resistance values for the three sizes was significant at the 5% level ($P < 0.05$) as determined by Tukey's test. The average resistance values at 5ºC were 6.96×10^5, 4.56×10^5 and 3.21×10^5 s/m for size 3L, L and M and the values at 20ºC were 5.74×10^5, 3.50×10^5 and 2.13×10^5 s/m for size 3L, L and M, respectively. Differences in resistance could be due to anatomical differences such as intercellular space volume. Intercellular space volume, which is associated with porosity, is a property of cultivars that depends on the growing season. Tissue with large porosity is generally assumed to provide an efficient means of aerating that could produce high gas diffusivity and low resistance within the tissue. In the present study, porosity did not significantly differ between the three fruit sizes ($P<0.05$) with 31.4, 32.1 and 33.7% for sizes M, L and 3L, respectively. Most studies on gas exchange in fruit and other bulky plant organs indicate that the skin represents the primary significant barrier to gas exchange between the product and the atmosphere surrounding it. Consequently, we considered that differences in resistance were caused by differences in their skin characteristics such as thickness, size of individual stoma and lenticel and density of stoma and lenticel.

To investigate the differences in skin characteristic among sizes, the relationship between skin characteristics (thickness) and fruit size was taken notice. As a result, the average skin thickness of sizes 3L, L and M were 6.72, 5.40 and 4.37 mm, respectively, and significant differences in thickness, resulting from albedo thickness, were determined by Tukey's test. The same relationship between skin thickness and fruit size was observed in Hamlin orange by MORGAN *et al.* (2005). Although the differences in individual size and density of stoma and lenticel must be considered, skin thickness can be the main characteristic of skin. Therefore, it was considered that the effect of fruit size on resistance was mainly caused by the skin thickness of fruit.

After deriving resistance values for ethane diffusion, it is important to know the resistance values for O_2 and CO_2, which are important in respiratory gas exchange. BANKS (1985) suggested that ethane diffusion is probably similar to O_2 and ethylene diffusion, because its movement is thought to be restricted to the gas phase, i.e., through stomata or lenticels. CO_2 diffusion, however, is suggested to be not necessarily equal; some CO_2 may also diffuse through the cuticle of fruit. PEPPELENBOS and JEKSRUD (1996) determined the relationship between the diffusion of various gases by using Graham's Law prediction, even though they described the difference in diffusion routes makes using only Graham's Law suspect. On the other hand, BEN-YEHOSHUA *et al.* (1985) showed that the resistance of Valencia oranges is similar for ethylene, CO_2 and O_2, whereas the resistance to water is 60-fold lower and suggested that the mass transport of ethylene, CO_2 and O_2 occurs by the same mechanism, which is different from water. These results assume that the mass transport of ethane, O_2 and CO_2 for citrus fruits, which has comparatively larger porosity, occurs in the same diffusion route and the resistance values of the gases are similar. Therefore, we used Graham's Law as proposed by PEPPELENBOS and JEKSRUD (1996) and proposed the next equations to predict the resistance values for O_2 and CO_2 by using the measured values determined with the ethane efflux method:

$$RO_2 = RE/0.968 \quad (8)$$

$$RCO_2 = RE/0.826 \quad (9)$$

RO_2, RCO_2 : Resistance for O_2 and CO_2 (s/m)
RE : Resistance for ethane (s/m)

(Conclusion) The determination of intercellular space volume (V_{in}) in eight species of citrus fruits and the development of a simple prediction method for V_{in}. For all eight species, V_{in} ranged from 9.2 cm^3 to 142.2 cm^3. For each species, it can be seen that size, mass, and total volume were proportional to V_{in}. The porosity of the eight species ranged from 6.7% to 29.4%. The real density of the species was almost similar to the mean value, 1.0647 g/cm^3. On the basis of the mean value, two mathematical models to predict V_{in} are developed. Based on four statistical evaluation methods, model $V_{in} = V_t - \frac{m}{1.0647}$, where V_t is total volume measured using Archimedes' principle and m is mass of a fruit, was evaluated and determined to be acceptable for predicting V_{in}. However, the alternative model, $V_{in} = V_s - \frac{m}{1.0647}$, where Vs is sphere equivalent volume calculated by average diameter, was not acceptable.

By modifying CAMERON and YANG's method, our results showed that the method was applicable for measuring resistance and reduced the time required to measure resistance. Further analysis showed that resistance was influenced by citrus size and temperature. The larger the size of fruit, the greater the resistance value. Although the differences in individual size and density of stoma and lenticel must be considered, skin thickness can be the main characteristic of skin. Therefore, it was considered that the effect of fruit size on resistance was mainly caused by the skin thickness of fruit. In addition, the higher the temperature, the lower the resistance value. It could be explained that gas diffusivity in the diffusion route from intercellular space to stomata or lenticels of fruit, gas phase diffusion, depends on temperature and increases in temperature.

The conversion to resistances for O_2 and CO_2 and their average values and variation resulted in a somewhat large variation that could be mainly due to skin thickness. However, this average value may be used to estimate the average resistance for other temperatures and sizes.

(**Anticipatory benefits**) The mathematical modelling for predicting intercellular space volume (V_{in}) can be simply applied to determine the V_{in} of citrus fruits. In addition, an improved method for measuring the resistance of citrus Iyo fruits (*Citrus iyo hort. Ex Tanaka*) to gas diffusion is suitable for measuring resistance and can shorten the time required to measure resistance. Therefore, it is considered that this method can be applied easily in measuring resistance of other citrus species.

References

Banks, N. H. (1985). Estimating skin resistance to gas diffusion in apples and potatoes. *Journal of Experimental Botany*, *36*(173), 1842–1850.

Ben-Yehoshua, S., Burg, S. P., & Young, R. (1985). Resistance of citrus fruit to mass transport of water vapor and other gases. *Plant Physiology*, *79*(6), 1048–1053.

Cameron, A. C., & Yang, S. F. (1982). A simple method for the determination of resistance to gas diffusion in plant organs. *Plant Physiology*, *70*(1), 21–23.

Dirpan, A., Hikida, Y., Chiba, H., & Ogawa, Y. (2015). Mathematical modeling of intercellular space volume of citrus fruits. *Food Preservation Science*, *41*(3), 103–110.

Ebrahim, R. M., Ishida, S., Tanaka, F., Uchino, T., Hamanaka, D., & Hikida, Y. (2014). Temperature dependency of gas diffusity and skin resistance of japanese pear ('oushuu') based on the fruit's true 3D geometry. *Food Science and Technology Research*, *20*(2), 247–253.

Knee, M. (1991). Rapid measurement of diffusion of gas through the skin of apple fruits. *Hort Science*, *26*(7), 885–887.

Morgan, K. T., Rouse, R. E., Roka, F. M., Futch, S. H., & Zekri, M. (2005). Leaf and Fruit Mineral Content and Peel Thickness of " Hamlin " Orange. *Proc. Fla. State Hort. Soc*, *118*, 19–21.

Peppelenbos, H. W., & Jeksrud, W. K. (1996). A method for the simultaneous measurement of gas exchange and diffusion resistance under various gas conditions. *Acta Horticulture*, *464*, 333–338.