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Effect of various citrus sizes on the resistance to gas diffusion

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Abstract

This study investigated the effect of various sizes of citrus on their resistance (R) to gas diffusion. The purpose of the investigation was to compare the value of R in 3 different sizes of citrus. To measure the R to gas diffusion in citrus, the study applied ethane efflux method. This is the method which the evolution phenomenon of ethane was measured by applying Fick's Law. The results showed that R of ethane (C_2H_6) gas was dependent on citrus size. It can be seen that the larger the size of the fruit, the greater the R value, i.e. M size had R=4.33x10^{5}s.m^{-1}, L size had R=4.99 x10^{5}s.m^{-1} and 3L size had R=6.84 x10^{5}s.m^{-1}.This finding indicated that the fruit sizes can be considered as an important factor in designing storage control atmosphere (CA) condition for citrus.

Keywords: Citrus Size; resistance; gas diffusion

1. Introduction

Citrus is the most widely produced fruit. It has many varieties and it grows in more than 80 countries[1]. 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. Citrus fruit production concerns for the sustainability challenges in the past, including pesticide use, post-harvest quality, and change of consumer preferences[2]. In this study, we focus on the post-harvest aspect.

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Nomenclature

ds/dt	amount of gas movement ($10^{-6}m^3.s^{-1}$)
A	surface area of fruit (m^2)
C_{in}, C_{out}	gas concentration in and outside product ($10^{-6}m^3.m^{-3}$)
t	time (s)
R	resistance ($s.m^{-1}$)
V_{in}	intercellular space volume (m^3)
k	negative slope of a plot of In (1- C_{out}^t/C^∞) versus t (s^{-1})
C^∞	concentration of ethanein the close box after equilibrium ($10^{-6}m^3.m^{-3}$)
V_t	total volume of fruit (m^3)
m	fruit mass (g)
$Ø$	porosity (%)
V_1, V_2	rate of gases diffusion ($m.s^{-1}$)
M_1, M_2	molecular weights of gases ($g.mol^{-1}$)
R_1, R_2	resistance of gases ($s.m^{-1}$)
P_a	apparent density of fruit ($g.m^{-3}$)

One beneficial effect of controlled atmosphere (CA) storage is to extend the postharvest life of fruit and vegetables. The precise temperature and level of O_2 and CO_2 are required to maximize storage life and to minimize storage defects. The empirical study on many fruits showed that oxygen can be lowered in CA storage ranging from 1% to 5% without subsequent defects [3].

The concentration limits for O_2 reduction and CO_2 enrichment in CA storage depends on the number of factors which include the respiration rate and the "tolerance" of the tissue toward lowered O_2 or increased CO_2. Meanwhile, one physical factor governing "tolerance" often left behind is the resistance (R) to gas diffusion[4].

The knowledge of R in gas transport properties 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[5]. Therefore, the understanding of resistance (R) in the CA storage is needed to predict permissible minimum gas levels in CA storage. It will also provide invaluable information of the physiological defect that may develop during 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, like CA storage. From the above explanation, it can be concluded that R in different sizes is an important factor to be investigated. However, information about the effect of fruit size on R is unavailable. Thus, the current research was initiated to study the effect of citrus size in three different sizes of citrus on R to gas diffusion.

2. Material and methods

2.1. Materials

Iyokan (*Citrus iyo Hort. ex Tanaka*) species in three different sizes (M, L, 3L) were used in this research. They were harvested on 27 January 2014 and stored in refrigerator for 9 week at 5^0 C during R measurement.

2.2. Methods of measuring resistance of ethane (C_2H_6)

The resistance to gas diffusion in citrus was determined by ethane efflux method[7]. Based on this method, single fruit was firstly placed in a jar purged with constant flow of air containing 1,800-2,000 ppm ethane. The duration required to reach the equilibrium was 5 hour (Fig. 1, step A). Next, the single fruit was transferred quickly in to another box (Fig. 1, step B). At the time of sealing, an AC fan attached to the box was turned on to ensure rapid mixing. Evolved ethane concentration from the fruit was measured by gas chromatograph at constant time interval. For picking the precise ethane, 0.5 ml gas samples were withdrawn at regular intervals through a septum attached to the box. After getting enough samples, the AC fan was turned off. The next 24 hour was used to let the whole system to come to equilibrium. The concentration of ethane in the jar was then re-measured using gas chromatography.

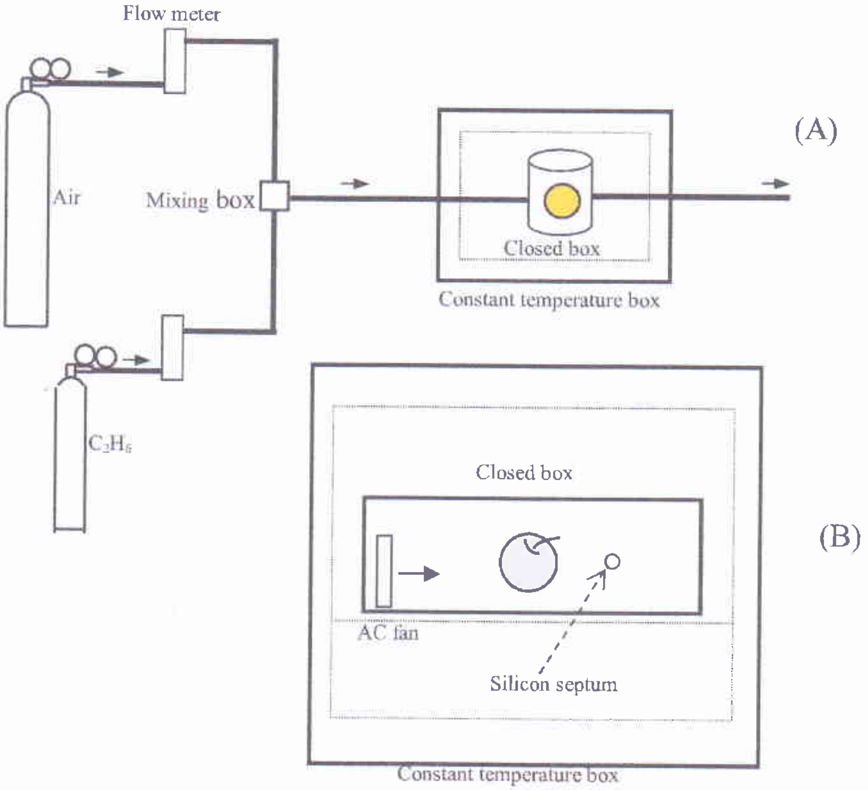

Fig.1 Schematic diagram of the experimental apparatus

Next, equation (1) was applied in the evolution phenomenon of ethane by applying Fick's Law.

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

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

$$R = \frac{A}{(V_{in})(k)} \tag{2}$$

surface area (A) was calculated from the citrus surface, assuming the citrus to be a perfect sphere. 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. The coefficient of determination (R^2) was found to be 0.999.

The measurements of R based on fruit sizes were performed at 5^{0}C for each size: M, L, and 3L respectively. All experiments were done in three replications.

2.3. Methods of measuring apparent density and porosity

The apparent density of whole fruit (P_a) is the ratio of fruit mass (m) and total volume (V_t) of whole fruit. Porosity (Ø) indicates volume fraction of void space or air in a material and is defined in equation (4)

$$Ø = \frac{V_{in}}{V_t} 100\% \tag{4}$$

2.4. Determination of Resistance for CO_2 and O_2

After obtaining resistance values for ethane in citrus fruits, it is important to calculate the R values for CO_2 and O_2. Using Graham's Law, predictions on the relationship between the rate of diffusion and R can be made[8]. Therefore, measuring the R value for ethane of the citrus and knowing molecular weight of gases permits us to predict the R values for CO_2 and O_2 respectively by using equation (5)[8,9]

$$\frac{V_1}{V_2} = \sqrt{\frac{M_2}{M_1}} \tag{5}$$

Since the inverse of rate gases diffusion (V_1,V_2) is resistance to gases diffusion (R_1,R_2), R values for CO_2 and O_2 can be calculated by re-arranging equation (5) into equation (6) or (7) respectively.

$$\frac{1/R_1}{1/R_2} = \sqrt{\frac{M_2}{M_1}} \tag{6}$$

$$\frac{R_2}{R_1} = \sqrt{\frac{M_2}{M_1}} \tag{7}$$

2.5. Statistical analysis

Resistance (R) values were analyzed by one-way analysis of variance (ANOVA) based on sizes while the significance of differences among means of R were determined using Tukey's test. The level of significance was set as $P<0.01$. To test a significant difference between measured k and predicted k, paired t-test was employed at $P<0.01$. SPSS software for windows version 16.0 (SPSS Inc., IL, USA) and Microsoft Excel 2010 were used to analyze the data.

3. **Results and discussion**

3.1. Effect of fruit sizes on the R to gas diffusion

Table 1 shows basic properties of Iyokan citrus such as mass (m), total volume (V_t), apparent density (P_a) and porosity (Ø). In the present study, the fruits were harvested at the same time, so the difference is limited to the natural differences between individual fruits. In general, result for porosity indicated that there was no relationship between the size of fruit and porosity. For example, size L had only 23.6% porosity while M had 28.9% porosity. This result is consistent with the report of Bauman and Henze[10] investigating small and large varieties of apples. They found that large kind of apple such as Boskoop had the lowest range of porosity. In comparison, other varieties such as potato, tomato, and apple have various porosity ranging from1-2%, 15-20%, 25-30%, respectively[11]. Other cultivars such as Asian pears (*Hosui* and *Kosui*), Nectarine (*Sunglo* and *Red Gold*), apple (*Braeburn* and *Cox Orange Pippin*), and cucumber have various porosity ranging from 1.7-2.5%[12], 4.0-8.0%[12], 14.1-17.4%[12], and 3.4%[13] respectively.

Table 1. Basic properties of Iyokan citrus in the examined sizes

Size	m[g]	V_t[10^{-6}m^3]	P_a[10^{-6}g/m^3]	Porosity [%]
M	155.4	205.47	0.76	28.9
L	212.4	261.23	0.81	23.6
3L	272.2	385.50	0.71	33.7

Porosity(Ø) is useful as an additional factor when estimating sensitivity to low levels of O_2 and /or high CO_2 concentration and determining tolerable concentrations for CA[10]. For example, Bauman and Henze[10] categorized porosity of cv. Cox Orange in three different levels of tolerance to CA: low level (<14%), mid-level (14.1-16%), and high level (> 16%). Thus, the values of porosity in Table 1 can be used to learn more the relationship between citrus porosity and its tolerance to CA. Accordingly, the optimum gas concentration around citrus fruit in CA can be created.

The surface area (A), intercellular space volume (V_{in}), negative slope of a plot of ln ($1- C_{out}^{t}/C^{\infty}$) versus t (k) and resistance value (R) in three different sizes of fruit are shown in Table 2. From the calculation, it can be seen that size, m, and V_t in Table 1 for each size appear to be proportional to V_{in} in Table 2. The difference of V_{in} in each size could occur because the V_{in} can be a property of the cultivar. It is also a function on the growing season and the number of the size of cells [5,14-16]. Differences in V_{in} could give contribution to the differences in internal atmosphere composition between cultivars[17].

Table2. R value of ethane to gas diffusion together with the values A, V_{in} and k in three different sizes of citrus

Size	A [10^{-4}m^2]	V_{in}[10^{-6}m^3]	k [10^{-4}s^{-1}]	R[10^5s.m^{-1}]*
M	163.7	59.5	6.4	4.33^a
L	200.2	61.7	6.5	4.99^b
3L	236.9	129.9	2.7	6.84^c

*Means of R followed by different letters are significantly different as determined by the Tukey test at $P < 0.01$

The highest R occurs in 3L size followed by L and M size. Moreover, Tukey's test revealed differences in the R values in different size. This suggests that R value significantly differs from the three fruit sizes ($P < 0.01$). Size differences in R 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[6].

Interestingly, our findings of the study provide mixed results. Fruit with M and L sizes support prior study by Rajapakse et al.[12]. They have low porosity and high resistance values. In contrast, cultivar with 3L size has high value of porosity and resistance. It is assumed that this different result is caused by two reasons. First, previous study employs different cultivars (apple, pear, and nectarine) which differ from this current study (using one cultivar, citrus). The difference between the cultivars taken as samples may cause differentiation in their skin characteristics such as thickness of the cuticle, number of open lenticels, surface cracks, and wax deposits. They have an effect on R [12,14]. In addition, most studies on gas exchange in fruits and other bulky plant organs indicate that the skin represents the primary significant barrier to gas exchange between the commodity and the atmosphere surrounding it [7,14,18,19]. Second, prior study uses one size for each cultivar while the present study employs the same cultivar with different sizes.

The correlation between R, A, V_{in} and k was investigated statistically. In this case, we proposed bivariate correlation method by using Pearson coefficient because this coefficient is better to be applied in data having scale as in Table 2. The results of bivariate correlation between variables were presented in Table 3.

Table 3. Correlation coefficient A, V_{in} and k to R value

		A	V_{in}	k
R	Pearson Correlation (r)	0.956^{**}	0.965^{**}	-0.957^{**}
	Sig. (2-tailed)	0.000	0.000	0.000

**. Correlation is significant at the 0.01 level

Table 3 showed that the relationship between R and each variable, A, V_{in} and k is 0.000 (<0.01). It implies that there was significant correlation among the three factors with R. Moreover, Pearson's correlation (r) ranging from 1 to -1 indicates the relationship between variables as well as direction of the relationship. When r is close to 1 or -1, it indicates significant correlation between the two variables. On the other hand, a correlation coefficient close to 0 implies little or no relationship between the two variables. Positive coefficient indicates parallel association (X increase, Y increase), and negative coefficient indicates unparallel association (X increase, Y decrease). Coefficient correlation can be interpreted as follows: 0.00-0.199=very low, 0.20-0.399=low, 0.40-0.599=moderate, 0.60-0.799 strong, 0.80-1.000=very strong. Referring to the interpretation, it can be concluded that the relationship between the three variables, A, V_{in} and k to R is very strong. While A and V_{in} have positive association, k has negative association.

Showing that size of fruit has strong association with surface area (A), mass (m), intercellular space volume (V_{in}), and total volume (V_t), we continue to investigate the extent of the relationship of those variables with k by employing Linear, Quadratic, S-curve and Power methods. k is dependent variable while A, m, V_{in} and V_t are independent variables. We found that linear method is the best-fitted regression between variables based on coefficient determination (R^2), and variable V_{in} showed the closest relationship with k (R^2=0.98). The result of this relationship was presented in Fig. 2.

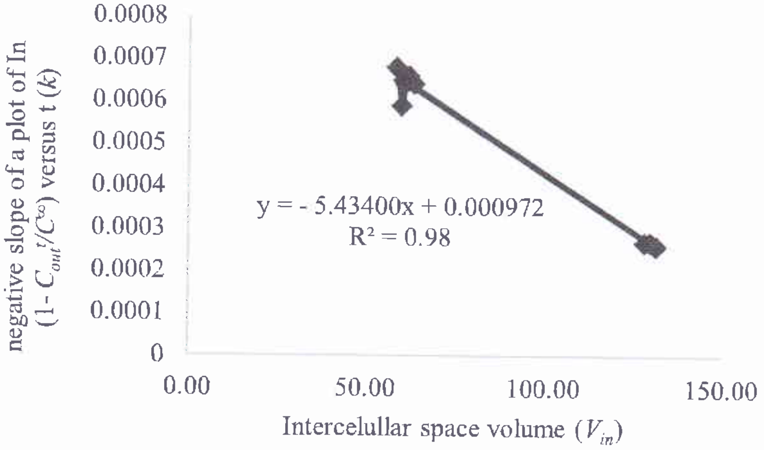

Fig. 2. The relationship between V_{in} and k

from Fig. 2, the equation y= -5.43400x+0.000972 can be re-arranged into equation (8)

$$k = -5.43400 V_{in} + 0.000972 \tag{8}$$

therefore, k can be predicted by using equation (8). To calculate one value of k, we need 24 hours to reach equilibrium condition including absorption time (approximately 5 hours) and sample injection with chromatography gas. It implies that calculation of the value is costly and time consuming.

To confirm the applicability of the equation (8), predicted values of k from equation (8) were compared to the actual values of k from experiment. The total of 3 samples were used to validate k value from equation (8) resulting values as follows 0.00037, 0.00036, and 0.00035. Meanwhile, the findings of experiment are 0.00034, 0.00030, and

0.00037. Then, paired t-test was used to determine whether there is a significant difference between the two measurements, prediction, and experiment. The result is 0.423 ($P<0.01$). It implies that statistically, there is no significant difference of k value either calculated using equation (8) or calculated using experiment. Therefore, equation (8) is appropriate to predict k based on variable V_{in}. However, it is suggested that the equation (8) is only valid to predict k within 5°C for Iyokan species.

3.2. Resistance for CO_2 and O_2

To predict R values for CO_2 and O_2 we need to know their molecular weight. Then, Graham Law can be used to obtain the R values for CO_2 and O_2[8]. In this term, we assumed that R C_2H_4 was similar to R C_2H_6. This issue has been proven by Cameron and Yang[7]. They found that there was a good agreement between R C_2H_6 and R C_2H_4 which indeed very similar. The results of R values for CO_2 and O_2 in three different sizes of citrus were presented in Table 4.

Table 4. R values of C_2H_6 to gas diffusion together with the prediction R values of O_2 and CO_2 in three different sizes of citrus fruit

Gases	Examined of R in three sizes $[x10^5\ s.m^{-1}]$		
	M	L	3L
C_2H_6	4.33^a	4.99^a	6.84^a
O_2	4.47^a	5.15^a	7.06^a
CO_2	5.24^b	6.04^b	8.28^b

*Means of R followed by different letters are significantly different as determined by the Tukey test at $P < 0.01$

Table 4 clearly demonstrates that there was significant difference in R values between C_2H_6,O_2 and CO_2 at M, L, 3L sizes. In addition, comparison of the mean R C_2H_6, O_2 and CO_2 indicated that R CO_2 and O_2 were higher than R C_2H_6. This result was consistent with previous study's finding by Dadzie[6] having R of eight apple varieties. The finding is that all apple varieties had higher R CO_2 than R C_2H_4. 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[6]. It is apparent that both O_2 and CO_2 would be expected to have higher R values than C_2H_6 since they move slower in the gas phase. On the other hand, this issue was not consistent with those reported by Ben-Yehoshua et al.[18] and Cameron and Yang[4]. They concluded that the mean R CO_2 and O_2 of citrus almost similar to R C_2H_6. They suggested that CO_2, O_2, and C_2H_4 move primarily through the holes of the fruits skin (stomates, lenticels) rather than thorough the cuticle. In other words, the mass transportation of fixed gases occurs in similar mechanism. Our R values are in line with the previous findings reported by Ben-Yehoshua et al.[18]. They found that the R CO_2, O_2, and C_2H_4 of citrus were $5.7x10^5$ $s.m^{-1}$, $6.0x10^5$ $s.m^{-1}$, $6.9x10^5$ $s.m^{-1}$ respectively. Hagenmaier and Baker[19] also reported that the R of CO_2 in citrus fruit was $6.5x10^5$ sm^{-1}.

The difference of R in the various sizes of citrus was related to the internal composition of O_2, CO_2, and C_2H_4[6]. The study by Dadzie[6] indicated that high R affects gas movement across the fruit skin and results in lower internal O_2 and higher CO_2 and/or C_2H_4 for a given respiration rate and external atmosphere composition. It is expected that the result of R differences between citrus sizes would respond differently to CA treatment. These findings are supported by other researchers including Cameron and Reid[4], Knee[20], Rajapakse et al.[12] and Dadzie[6]

4. Conclusion

Overall, this study gave proof quantitatively of the research. It showed the dependence of Resistance (R) to gas diffusion of Iyokan citrus to citrus size. It is the R depending on size of the citrus. Size differences of R to gas diffusion are likely to be important factor for determining sensitivity to CA limits. Knowledge of R might be used in collaborating with other physiological data to predict optimum CA storage conditions for citrus.

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