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A Comparative Study of Respiratory Activity of Tropical Products under Two Storage Conditions

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ABSTRACT

This study aimed to investigate respiration process of Indonesian tropical products and its parameter to support the use of CAS. Shallot, dragon fruit and sneak fruit that are high-value and export-potential products in Indonesia were investigated. For respiration measurement, the fruits were kept in tightly closed jars. The ratio of fruit volume and free volume of jar (headspace) was determined to calculate the rate of fruit respiration. To observe the storage condition effects, the jars were stored in two different temperatures: low temperature (7±2°C) and room temperature (27±2°C). In cold temperature storage, changes in O_2 and CO_2 concentrations are slower than in room temperature storage. The rate of O_2 consumption and CO_2 production of products during storage decreased as the O_2 concentration decreased for all conditions. Based on the dramatic increase of RQ value at low O_2 concentrations, the low oxygen limits (LOLs) of shallot, sneak fruit and dragon fruit were estimated at around 7.5%, 4% and 2% O_2 respectively, at the room temperature. However, the LOL was not detected yet at a cold temperature for 200 h of measurement due to a slow decrease of O_2. The results showed that different products had different respiration activities so that the storage procedures should be different. A determination of model-based LOL and validation would be needed in the next research to be precisely applied on CAS.

1. INTRODUCTION

Horticultural products such as fruits and vegetables are highly perishable due to their high respiratory activity after harvesting (Sukmawaty *et al*., 2019). Respiration in agricultural products is the continuous process of using oxygen (O_2) to break down complex molecules (carbohydrates) into simpler molecules such as carbon dioxide (CO_2), water vapor (H_2O), and energy (Fonseca *et al*., 2002). A common method to reduce respiration rate and extend shelf life is through cold storage (CS). However, better storage techniques are required for long-term storage to anticipate harvest failures, control prices, and cope with long-distance distribution chains such as exports.

Controlled atmosphere storage (CAS) is a modern non-chemical storage technique that is superior to CS (Ma *et al*., 2019). In recent years, CAS has been implemented on industrial scales in Indonesia. However, its application has not been extensively tested scientifically for local fresh products. The principle of CAS is to reduce the product's respiration rate by lowering the concentration of O_2 and slightly increasing the concentration of CO_2 in the storage space, combined with cooling (Peppelenbos, 2003; Boeckx *et al*., 2019). With a similar principle, modified atmosphere packaging (MAP)

is a cheaper and easier alternative to CAS. The gas in the package headspace is modified using absorbers (Nugraha *et al*., 2015) or by exploiting the packaging permeability.

Ideally, the O_2 concentration in CAS storage space or MAP headspace should be reduced to slightly above the low oxygen limit (LOL), which is the safe threshold where respiration changes to fermentation occur when O_2 is below the LOL level (Thewes *et al*., 2015). The safe limit or LOL value varies for each product depending on cultivar, harvesting time, product shape, and physical size (Bessemans *et al*., 2016). This makes it difficult to determine the optimal and safe atmospheric gas composition for CAS or MAP applications. Currently, information regarding the safe gas composition limits for CAS and MAP applications is limited for tropical products originating from Indonesia. This research aims to determine the respiratory characteristic of local fresh products with high export value and to establish the safe limit or LOL for each product for storage applications involving oxygen reduction, such as CAS and MAP.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this research were shallots (*Allium cepa*), snake fruit (*Salacca zalacca*), and fresh dragon fruit (*Selenicereus costaricensis*). Shallots of the Demak variety, recently harvested, were obtained from the traditional Beringharjo market in Yogyakarta. Fresh and physically intact shallots were used as samples. Snake fruit of the Pondoh variety with a ripeness level of 60 - 70% was obtained from the *salak* farmers association CV. Mitra Turindo, Turi, Sleman. Red dragon fruit with a ripeness level of 80% was obtained from Krajan, Jambewangi, Sempu, Banyuwangi. The samples were then transported to the Food Engineering and Postharvest Laboratory, Department of Agricultural and Biosystems Engineering, Faculty of Agricultural Technology, Universitas Gadjah Mada for measurements. During transportation, the product quality was maintained by placing them in containers/boxes to prevent exposure to environmental air, which could accelerate the deterioration process.

2.2. Observation Procedure

The samples that have arrived at the laboratory were weighed and their volumes are measured. The sample weights used for respiration were approximately 300 grams for shallots, 500 grams for snake fruit, and 500 grams for dragon fruit. Additionally, the total volume of the jar containers (1.675 L) used for measuring respiration, as well as the free volume or headspace, were determined by measuring the difference between the total volume of the jar container and the volume of the product inside it. The free volume or headspace for shallots was 1355 ml, snake fruit was 1165 ml, and dragon fruit was 1321 ml. The lid of the jar was perforated, and a rubber septum was provided for taking gas samples which were then analyzed using a gas analyzer (902D DualTrak, USA). The jar containers were then tightly sealed and locked to ensure no gas leakage from the environment into the jar containers, which could affect measurement results. The jars were then stored at two different temperature conditions: low temperature (7±2 °C) and room temperature (27±2 °C), except for dragon fruit, which was only measured at room temperature. Measurements were repeated 3 times for each sample.

Figure 1. The experimental setup for measuring the respiration of shallots, snake fruit, and dragon fruit

The oxygen (O_2) and carbon dioxide (CO_2) gas composition in the jars was periodically observed using a gas analyzer. Gas samples were taken for 200 h at varying intervals, namely: 1-h intervals for the first 12 h, 8-h intervals for 60 h, 12 h intervals from the 32nd to the 144th h, and 24 h intervals up to the 200th h (until the change in gas concentration was constant).

2.3. Data Analysis

2.3.1. Respiration Rate

The respiratory process is a metabolic activity that provides the energy needed for biochemical reactions within fresh produce. The respiratory reaction is generally expressed as the following (Patel *et al*., 2016):

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 673 \text{ kcal} \quad (1)$$

The respiration rate indicates the length of the shelf life of a horticultural product. The respiration rate of a product is known based on changes in the concentrations of O_2 and CO_2. The respiration rate of a horticultural product can be calculated using the following equation (Ho *et al*., 2008):

$$\frac{\partial C_{O_2}}{\partial t} = \frac{m_p}{V_t - V_p} \cdot R_{O_2} \quad (2)$$

$$\frac{\partial C_{CO_2}}{\partial t} = \frac{m_p}{V_t - V_p} \cdot R_{CO_2} \quad (3)$$

The symbol ∂C_{O_2} and ∂C_{CO_2} respectively represent changes in the concentration of O_2 and CO_2 (%), ∂t represents changes in time (h), V_p and V_t respectively represent the volume of the measured fresh product and the volume of the measurement container (ml), m_p represents the mass of the product (kg), R_{O_2} represents the rate of oxygen consumption (ml.kg^{-1}.h^{-1}), and R_{CO_2} represents the rate of CO_2 production (ml.kg^{-1}.h^{-1}). Equations (2) and (3) are formulated to calculate the respiration rate of fresh products (oxygen consumption and CO_2 production) from the measurement results as described in the following Equations (4) and (5):

$$R_{O_2} = \frac{(C_{O_2,i} - C_{O_2,f}) \cdot V_h}{100 \cdot m_p \cdot (t_f - t_i)} \quad (4)$$

$$R_{CO_2} = \frac{(C_{CO_2,f} - C_{CO_2,i}) \cdot V_h}{100 \cdot m_p \cdot (t_f - t_i)} \quad (5)$$

where subscripts *i* and *f* indicate the initial and final conditions of gas concentration data collection and measurement time, V_h represents the headspace volume or remaining empty space inside the container (ml), obtained from the difference between V_t and V_p.

2.3.2. Respiratory quotient

Respiratory quotient (RQ) is expressed as the ratio of the rate of CO_2 production to the rate of O_2 consumption. The value of RQ was calculated using the following equation (Peppelenbos *et al*., 1996; Saenmuang *et al*., 2012):

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \quad (6)$$

The RQ value typically ranges between 0.7 and 1.0. If the RQ exceeds 1, or the rate of CO_2 production is greater than the rate of O_2 consumption, the fresh product is indicated to undergo fermentation processes (Saenmuang *et al*., 2012).

2.3.3. The Michaelis-Menten Model.

The known Michaelis-Menten respiration model is used to describe the respiration process of fresh products to predict the respiration rate. When the gas concentration also changes over time, the measured respiration rate and the predicted

respiration rate over time are compared. The non-competitive inhibition type respiration model is used to describe the rate of O_2 consumption and CO_2 production using the following equation (Peppelenbos *et al*., 1996):

$$R_{O_2} = \frac{V_{m,O_2} \cdot C_{O_2}}{(K_{m,O_2} + C_{O_2})\left(1+\frac{C_{CO_2}}{K_{mn,O_2}}\right)} \quad (7)$$

where V_{m,O_2} is the maximum oxygen consumption rate (ml.kg^{-1}.h^{-1}), C_{O_2} is the oxygen concentration (%), C_{CO_2} is the carbon dioxide concentration (%), K_{m,O_2} is the Michaelis-Menten constant for oxygen consumption (%), K_{mn,O_2} is the Michaelis-Menten constant for non-competitive CO_2 inhibition (%), and R_{O_2} is the O_2 consumption rate (ml.kg^{-1}.h^{-1}).

$$R_{CO_2} = RQ_{ox} \cdot R_{O_2} + \frac{V_{m,f,CO_2}}{\left(1+\frac{C_{O_2}}{K_{m,f,O_2}}\right)} \quad (8)$$

where V_{m,f,CO_2} is the maximum carbon dioxide production rate (ml.kg^{-1}.h^{-1}); K_{m,f,O_2} is the Michaelis-Menten constant for oxygen inhibition on fermentative CO_2 production (%); RQ_{ox} is the RQ value at high oxygen concentration range, and R_{CO_2} is the carbon dioxide production rate in the sample (ml.kg^{-1}.h^{-1}).

3. RESULTS AND DISCUSSION

The decrease in O_2 concentration and the increase in CO_2 concentration inside the jar occurred more slowly at low temperature (7±2 °C) compared to room temperature (27±2 °C). At the same measurement temperature, the changes in gas concentration also varied depending on the fresh product (Figure 2). At low temperature, both O_2 and CO_2 concentrations in the jar containing shallots only changed by a few percent (±1%) from the initial to the final measurement time while the gas changes were greater at room temperature (Figure 2A). O_2 and CO_2 concentrations reached 5% and 15%, respectively, in 100 h before the gas levels became constant.

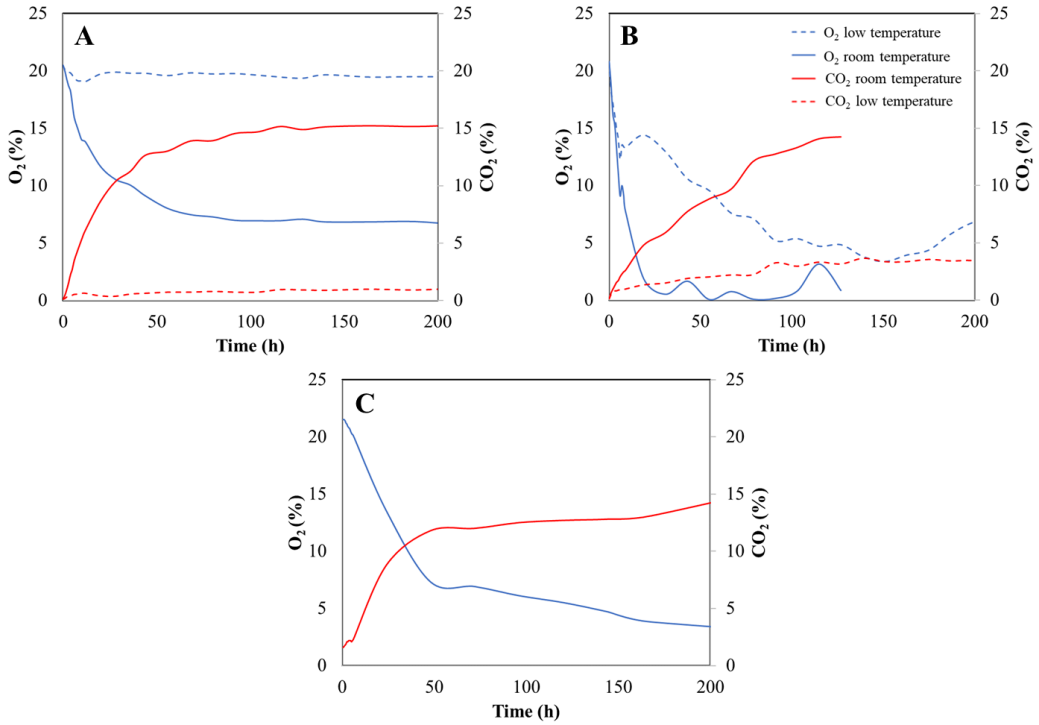

Figure 2. The changes in O_2 and CO_2 concentrations (%) in the headspace over time (h) as the respiratory activity of shallots (A), snake fruit (B), and dragon fruit (C) at low temperature (7±2 °C) and room temperature (27±2 °C).

Snake fruit underwent a faster decrease in O_2 compared to other fresh products, both at low and room temperatures (Figure 2B). The O_2 concentration of snake fruit declined to ±5% within 100 h at low temperature and, even, to ±1% within 20 h at room temperature. Interestingly, although the CO_2 concentration of snake fruit increased throughout the measurement, it did not ideally mirror the O_2 concentration profile, as commonly shown in O_2 and CO_2 concentration measurement. The CO_2 increase in the jar was slower than the O_2 decrease. At room temperature, the O_2 and CO_2 concentrations of dragon fruit changed similarly to the gas profiles of shallots (Figure 2C).

O_2 concentration decrease and CO_2 concentration increase over the measurement time indicated the respiratory activity of fresh products, which were clearly distinguished by product type and temperature. Hertog *et al.*, (1998) explained that respiratory activity is influenced by enzyme reactions, depending on the storage temperature. At low temperatures, enzyme reactions within cells will decrease, resulting in a slow respiration rate (Sudjatha & Wisaniyasa, 2017). Slower changes of O_2 and CO_2 during low-temperature respiration measurements were also previously found by Rahayu *et al.*, (2021). In addition to temperature, structural diversity contributes significantly to the rate of O_2 intake and CO_2 production, even in the same product variety, as explained by Ho *et al.* (2018) and Nugraha *et al.*, (2022). The different rates of O_2 consumption and CO_2 production in snake fruit may be due to the peel structure of the snake fruit, which has different outer and inner surface contours. With the ribbed peel, O_2 can more easily enter the product (consumed) while CO_2 produced from respiration accumulates in the air spaces between the fruit flesh and peel before diffusing out. This results in a considerable difference in the CO_2 production and O_2 consumption rates of snake fruit, especially at initial measurement times or high O_2 concentrations (Figure 3B and 4B), also leading to very low average of RQ values for snake fruit (< 0.5) (Figure 5B). These indicate that post-harvest handling for different product types will vary according to their structure and thus physiological activities.

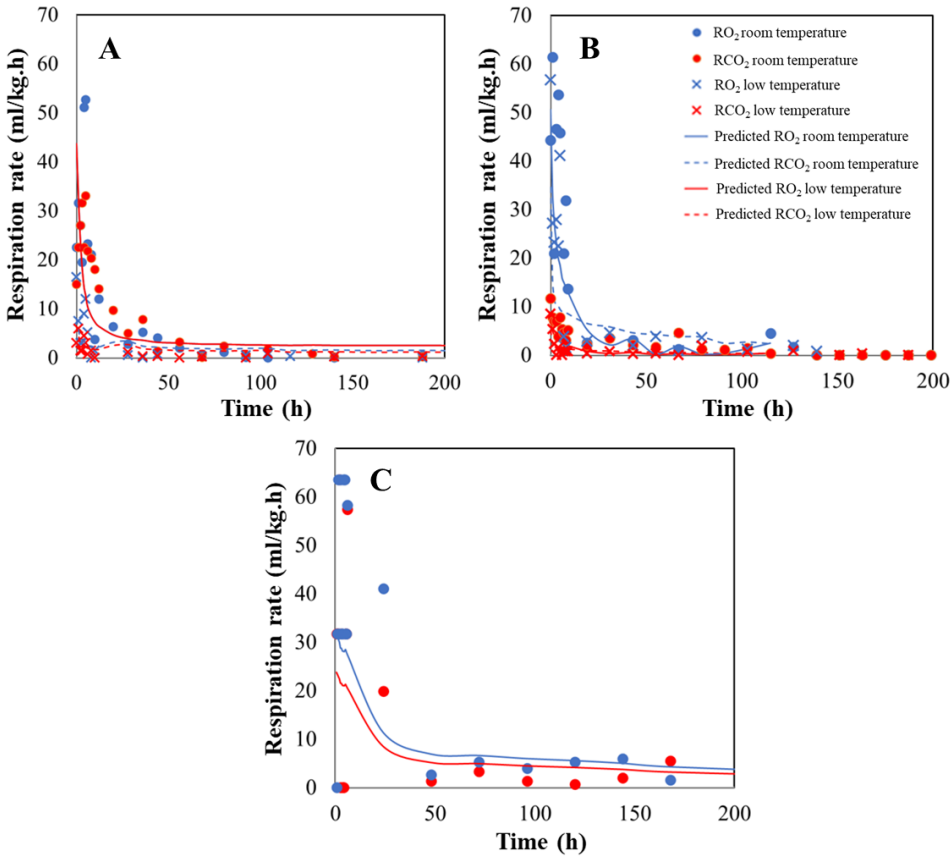

Figure 3. The changes in the rate of oxygen consumption (RO_2) and carbon dioxide production (RCO_2) over time (h) in shallots (A), snake fruit (B), and dragon fruit (C) at low temperature (7±2 °C) and room temperature (27±2 °C). Solid and dashed lines are predicted using Michaelis-Menten models.

Table 1. Respiration parameters of shallots, snake fruit, and dragon fruit under different conditions.

Product	Temperature (ºC)	Vm.O_2 (ml.kg^{-1}.h^{-1})	Km.O_2 (%)	Kmn.CO_2 (%)	RQ_{ox}	Vm.f.CO_2 (ml.kg^{-1}.h^{-1})	Km.f.O_2 (%)
Shallots	7	16.56	1.00	0.10	0.80	0.44	1.00
	27	52.70	1.00	1.80	1.00	0.44	0.05
Snake fruit	7	55.59	1.50	0.20	0.15	0.83	0.05
	27	60.29	1.50	0.90	0.15	1.53	0.00
Dragon fruit	27	63.56	8.00	3.50	0.75	19.00	0.00

The rate of O_2 consumption and CO_2 production changed throughout the measurement time. Figure 3 shows high respiration rates at the initial measurement period (first 12 h), then decreased to a constant level at subsequent measurement periods. In general, shallots had lower respiration rates at the intial times than the other products (Figure 3A). At room temperature, dragon fruit experienced a longer period of high respiration rate (up to 40 h) compared to shallots and snake fruit (Figure 3C). The decreasing respiration rate over the time corresponded to the decreasing availability of oxygen in the jar headspace (Figure 2). Michaelis-Menten models (Equations 7 and 8) were generally able to predict the rate of O_2 consumption and CO_2 production with the model parameters presented in Table 1. Michaelis menten constants (K_{m,O_2}) did not vary with the temperature difference but affected by the product sizes. Ho et al. (2013) explained that K_{m,O_2} is clearly in line with the product geometry scale, spatially incorporating all the diffusion resistance effects such as length of diffusion route and tissue complexity. The larger fresh product size is, the higher K_{m,O_2} value becomes. Critical or low oxygen limit of apple has been known to be approximately 46-78% of K_{m,O_2} (Ho et al., 2013). However, the limit will be different for shallot, snake fruit, and dragon fruit. Bessemans et al. (2016) reveals that the limit varies depending on the product, cultivar, harvesting batch, and product size.

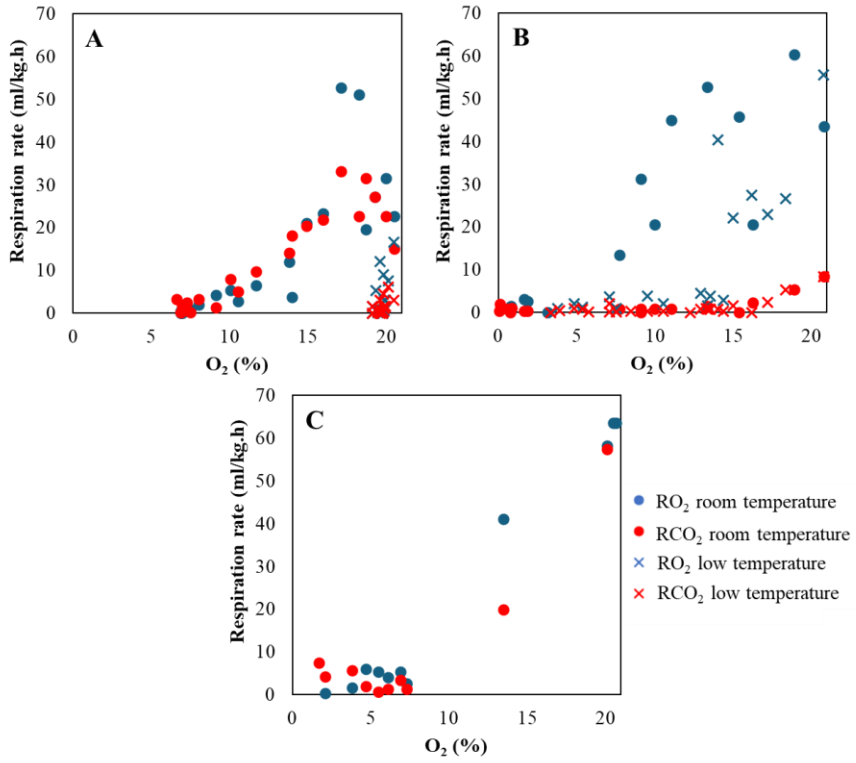

Figure 4. The changes in the rate of oxygen consumption (RO_2) and carbon dioxide production (RCO_2) in response to changes in O_2 concentration in shallots (A), snake fruit (B), and dragon fruit (C) at low temperature (7±2 °C) and room temperature (27±2 °C).

At certain times when the O_2 concentration in the jar becomes lower, the rate of O_2 consumption and CO_2 production ($ml.kg^{-1}.h^{-1}$) decreased further, as shown in Figure 4. At low temperatures, the respiration rate of the products was very low even at high O_2 concentration (21%). This is related to the availability of O_2 in the mitochondria within the cells, which determines the electron transport in the cellular respiration process (Ho *et al*., 2020). The difference in the rate of O_2 consumption with respect to O_2 concentration at low temperatures was not clearly observed in low-temperature measurements, both in shallots and snake fruit. This is because temperature has the strongest influence on the respiratory process, followed by low O_2 concentration and high CO_2 concentration (Hertog *et al*., 1998; Boeckx *et al*., 2019). In addition to being influenced by O_2 concentration and storage temperature, the respiration rates were also different in different fresh products. Ho *et al*., (2020) explained that the availability of O_2 in cell mitochondria depends on gas diffusion from the environment to the mitochondria. It is influenced by the macro and micro anatomy of the products as gas transfer routes (Mebatsion *et al*., 2008; Herremans *et al*., 2014). Differences in macro and micro structures in the products are indicated to play a role in determining the respiration of fresh products, and the vulnerability of products to damage as explained by Ho *et al*. (2018) and Nugraha *et al*., (2022) for pears. This also confirms that the storage procedures for each product are not the same and require more specific treatments for each product.

The ratio of carbon dioxide production rate to oxygen consumption rate, expressed as RQ, varied over time, storage temperature, and product type (Figure 5). In shallots, the RQ values fluctuated from time 0 to the end, and the RQ values at room temperature were generally higher than those at low temperature (Figure 5A). The RQ in shallots had a range of values from 0 to 2 with some points having higher values. At both room and cold temperatures, the RQs in snake fruit were relatively lower compared to shallots, with a range of average values < 0.5 (Figure 5B). As explained earlier, difference in respiration gas production and consumption rates affect the RQ value. The RQ values in dragon fruit ranged from 0 to 1.5 at the initial of the measurement, becoming relatively constant (< 1) in subsequent measurements and increasing dramatically at the end of the measurement period (Figure 4C). The difference in the range of RQ values also indicate that each product has a different safe limit range. High RQ values (exceeding the safe limit) in the storage room headspace are previously utilized as indicators of fermentation occurrence when the rate of CO_2 production exceeds the rate of O_2 consumption as a result of the fermentation process (Bessemans *et al*., 2016; Nugraha et al., 2022).

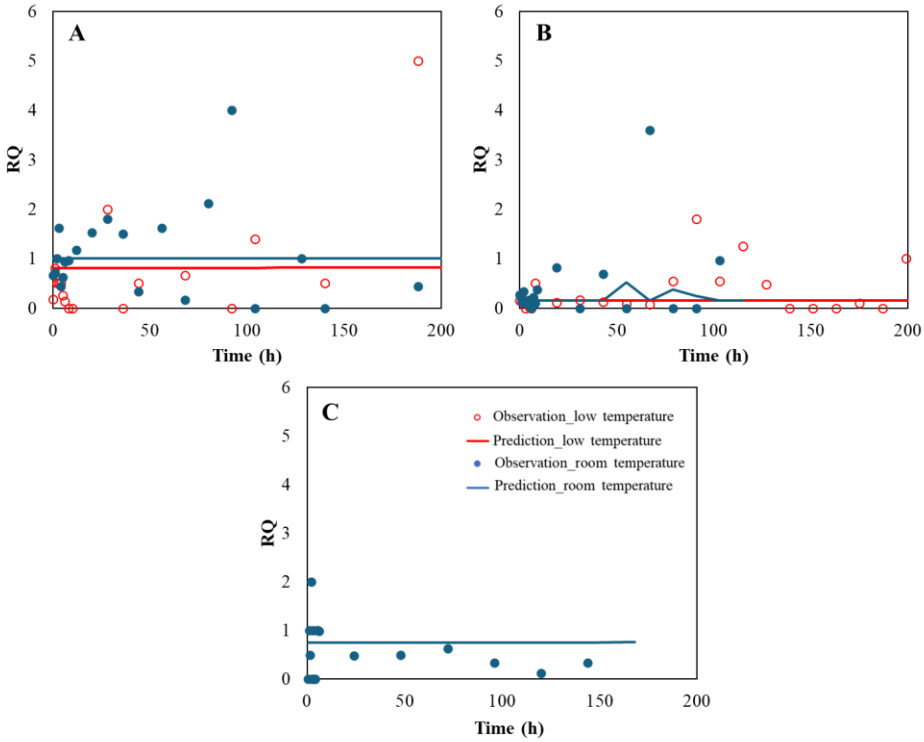

Figure 5. The values of Respiratory Quotient (RQ) over time (h) in shallots (A), snake fruit (B), and dragon fruit (C) at low temperature (7±2 °C) and room temperature (27±2 °C).

Regarding the relationship between RQ and O_2 concentration, overall, RQ did not significantly increase at low oxygen conditions (hypoxia, < 21%) at low temperatures, both in snake fruit and shallots (Figure 6A). At room temperature, the RQ values for all three products increased at low O_2 concentrations, with varying starting points of the increase (Figure 6B). The increase in RQ values approximately started at 7.5% O_2 for shallots, 4% O_2 for dragon fruit, and 2% O_2 for snake fruit, indicating the low oxygen limit (LOL) for each product. LOL can be defined as the transition boundary between aerobic respiration and fermentation (Wright *et al*., 2010). Below the limit, aerobic respiration process may switch to fermentation, producing low energy for cell maintenance. For a long time, the insufficient energy will cause tissue breakdown, generally known as internal browning incidence. The variation in LOL for each product indicates different sensitivities of the products when stored under low oxygen conditions such as in controlled atmosphere storage (CAS) or in modified atmosphere storage (MAP) applications. The gas concentration should be set slightly above the critical limit to avoid internal browning

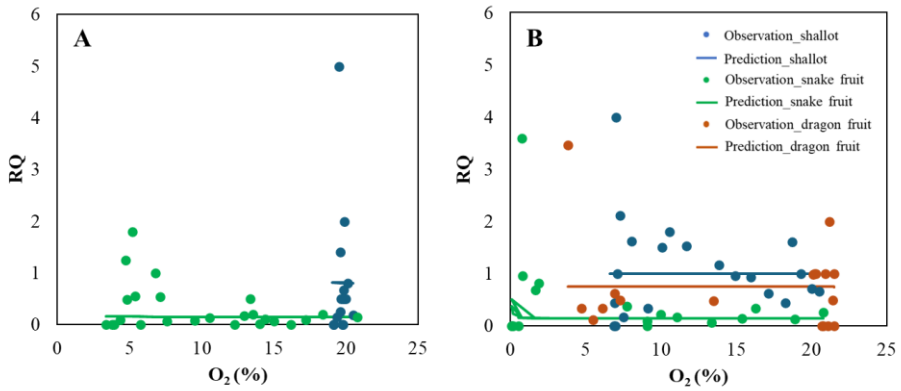

Figure 6. The calculated Respiratory Quotient (RQ) values in the respiration of shallots, dragon fruit, and snake fruit at low temperature (A) and room temperature (B).

In this study, the predictive model of RQ values was not yet fully capable of detecting an increase in RQ at low O_2 levels (Figure 6B). The increase in predicted RQ under hypoxic conditions was only observed for snake fruit at room temperature. At low temperatures, the absence of an increase in RQ values at low hypoxic conditions was due to the decrease in headspace O_2 (due to product consumption) not yet reaching the LOL value, as already achieved in room temperature measurements. Although the RQ values in the headspace were still within safe limits (not indicating fermentation), this did not necessarily reflect the conditions inside the products. Previously, Nugraha *et al*., (2022) found high RQ values, exceeding the safe limits, inside pears due to the resistance of gas exchange by the skin and the complexity of the product's microstructure.

CONCLUSIONS

The results of this study indicate that different fresh products have different respiratory activities, suggesting different storage procedures for each product. When stored at high temperatures, an increase in respiration occurs, indicating that low-temperature storage effectively reduces respiration rates. Determining safe limits for local fresh products under low oxygen conditions is necessary for future research so that storage using CAS or MAP can be optimized. The low oxygen limits (LOL) observed in this study were 7.5% O_2 for shallots, 4% O_2 for dragon fruit, and 2% O_2 for snake fruit. More accurate method to determine the LOL should be done in the future with incorporating the respiratory gas exchange simulation to know the internal concentration of gases. Additionally, gas exchange computations in salak are also needed to address the differences in oxygen consumption and carbon dioxide production.

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