How respiratory gas diffusivity correlates with porosity of plant organ tissues

B Nugraha1,3, P Verboven1, S Janssen1 and B Nicolaï1,2
1BIOSYST-MeBioS, KU Leuven, Willem de Croylaan 42, 3001 Leuven, Belgium
2Flanders Centre of Postharvest Technology, Willem de Croylaan 42, 3001 Leuven, Belgium
3Agricultural and Biosystems Engineering Department, Universitas Gadjah Mada, Jl. Flora No.1, Bulaksumur, Yogyakarta 55281, Indonesia

E-mail: bayu.nugraha@kuleuven.be or bayu.nugraha@ugm.ac.id

Abstract. Gas diffusion in bulky plant organs is relevant for understanding the respiratory metabolism. Limited tissue diffusivity due to low porosity may create gas concentration gradients leading to hypoxia. As porosity is known to be different between and within different tissues, oxygen (O2) diffusivity cannot be regarded as a homogeneous gas transport parameter of plant organs. This study aims to study the relationship between O2 diffusivity and porosity for three different horticultural products: eggplant, apple, and turnip, which have shown variability in tissue microstructure. Based on X-ray CT images, porosity maps of the whole fruit and vegetable have been previously created. O2 diffusivity was computed using a microscale model solved on the corresponding 3-D tissue microstructure of different samples across the product, derived from high resolution X-ray CT scan. The correlation between O2 diffusivity and porosity was determined along the radial tissue sample, as affected by region of interest (ROI) size. The correlation was strong in eggplant tissue ($R^2 = 0.95$), compared to the other products. Distinct distribution of the O2 diffusivity to the porosity was influenced by the physical characteristic of tissue intercellular space. The reduced ROI size changed the O2 diffusivity-porosity relationship, caused by the change in physical structure of the tissue. In conclusion, the tissue diffusivity did not linearly link to the porosity, other microstructural parameters and the size of the considered tissue sample affected the correlation.

1. Introduction

Tissue porosity of horticultural products varies depending on the tissue location or types of product [1-3]. Low porosities in certain tissue location can be the factor of dropped respiratory gas diffusivities [4,5]. It potentially causes local hypoxia or anoxia phenomena inside the product eventually triggering the symptom of internal breakdown. A multiscale gas exchange model was used to predict distribution of internal gas concentration in an entire apple [4]. However, since diversity in tissue microstructure has been known, respiratory gas diffusivity could not be considered as a homogeneous gas exchange parameter in an entire plant organ. Limited measurement methods make a comprehensive gas diffusivity calculation or measurement in each tissue position of a whole fruit or vegetable organ unavailable to date.
In earlier study, three-dimensional porosity distribution in eggplant, apple, turnip and pear organ has been mapped by utilizing X-ray CT images [3]. Grayscale intensity of the sample CT images was correlated with the tissue porosity calculated at identical position to generate a grayscale-porosity equation model. The strong correlation model was used to map tissue porosity of an entire horticultural product by only utilizing the grayscale values of juiced sample (0% porosity) and air or image background (100% porosity).

To transform the porosity values in the entire horticultural products into oxygen (O$_2$) diffusion coefficients, correlation between tissue O$_2$ diffusivity and porosity was explored in this study. Three different samples: eggplant, apple, and turnip, having distinct tissue micro-architecture were used. Different sizes of region of interest (ROI) were investigated to find out the best diffusivity-porosity relationship. The observed correlation model will be utilized to generate O$_2$ diffusivity maps in a whole fruit or vegetable organ.

2. Material and method

2.1. Samples
‘Purple-globe’ eggplant, ‘Jonagold’ apple, and ‘top-purple’ turnip were collected from a market around Leuven and immediately moved to the laboratory. These products have variability in micro-morphology feature. The product selection was based on absence of physical defects.

2.2. X-ray computed tomography and image processing
In order to obtain the real tissue microstructure as an input geometry, a cortex tissue of each product was radially excised using a cork bore and scanned at high resolution (Figure 1). Eggplant and turnip tissues were imaged at 4.87 µm using a Skyscan (Bruker micro CT) machine when apple tissue was scanned at 4.00 µm using a Phoenix Nanotom-m system (GE Inspection Technology). At these resolutions, cells and pores of the tissues could be clearly visualized. A multiple-scan mode was set to scan a long radial tissue sample part by part. The radiographic tissue images were reconstructed using NRecon (Bruker micro CT) and Octopus Reconstruction (Octopus Imaging Software) to produce sequential slices of X-ray CT images. The reconstructed images were then transferred to Avizo 9.4 (VSG) for image processing.

A region of interest (ROI) was firstly determined along the radial tissue images to exclude the damaged tissue due to sampling. Secondly, noises on the selected CT images were filtered using a Median filter method. Thirdly, cell and pore fractions of the tissue represented by grey and black pixels were segmented using Otsu’s thresholding method to binarize the images. Finally, these binarized images were imported to Matlab 18.a (The Mathworks, Inc) for further analyses.
2.3. Correlation analysis
The binarized tissue images were divided into multiple cubical ROIs at different positions to represent variability in the tissue microstructure (Figure 1). 1.1x1.1x1.1 mm³ was the initial size of ROI that was then reduced to be 0.2x0.2x0.2 mm³ in order to study effect of ROI size to the correlation. Small ROI samples were derived from the center part of the big ROI sample. Subsequently, effective O₂ diffusivity and porosity were computed at each position and size of ROI. A microscale gas transport model was incorporated into the 3-D tissue geometry by means of voxel-based finite volume method to solve effective O₂ diffusivity [4]. Tissue porosity (%) was a ratio of pore and total tissue volumes multiplied by 100%. Finally, correlations between O₂ diffusivity and porosity were explored.

2.4. Microstructural analysis
Pore microstructure of each product that has identical porosities was analyzed and visualized to reveal O₂ diffusivity values that were not in line with tissue porosity. The calculated variables were the number of intercellular space, branching number and tortuosity. Tortuosity is a geometrical parameter that contributes to large extent to transport characteristics of porous media. It was ratio of actual pore length and straight pore length between two geometrical boundaries [6]. The higher tortuosity, the more resistant respiratory gases diffuse through the tissue.
3. Results and discussion

Correlations between O$_2$ diffusivity and porosity were different among the bulky plant products and changed with the size of ROI (Figure 2). In general, O$_2$ diffusivities followed the change of porosity values at higher porosity interval (> 43%) while some diffusivity values were out of the regression line at lower porosity range. Eggplant tissue that has a wide range of porosity showed the strongest diffusivity-porosity correlation with big ROI size ($R^2 = 0.93$), compared to turnip and apple (Figure 2A). A similar porosity range was exhibited on the turnip tissue. However, the distribution pattern of O$_2$ diffusivity values to the porosity was different ($R^2 = 0.79$) (Figure 2B). It was wider at identical porosity points. In apple, air fraction of the tissue which was lower than 43% weakly correlated with effective O$_2$ diffusivity, shown by a low coefficient of determinant, $R^2 = 0.23$ (Figure 2C). Distinct physical feature of the intercellular spaces on each plant tissues likely contributed to large extent in the built correlations. It plays a role in facilitating gas transport mechanisms in plant organs [4,7].

The reduced ROI size changed the distribution of O$_2$ diffusivity values to porosity and improved coefficients of determinant ($R^2$) for all products (Figure 2E, F, and G). The altered correlations with small ROI were more likely caused by the simplification of pore structure complexity of the tissues, as shown in Figure 3. Very low O$_2$ diffusivities at low porosity range were affected by liquid fraction (cells) dominating the tissue at the considered ROI size prior to drastically increasing at higher porosity ranges. Gas diffusion coefficients are lower in liquid phase than in air (intercellular space) [4]. In contrast, High O$_2$ diffusivity

Figure 2. Correlations between O$_2$ diffusivity (m$^2$/s) and porosity (%) on eggplant (A-E), turnip (B-F) and apple (C-G) with 1.1x1.1x1.1 mm$^3$ (top) and 0.2x0.2x0.2 mm$^3$ (bottom) ROI sizes.
values at limited porosity range and low O\textsubscript{2} diffusivity at higher porosity interval could be caused by the trait of local pore structures such as tortuosity and connectivity between two geometrical boundaries that were perpendicular to the considered direction of gas diffusion.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Three-dimensional pore structure of the eggplant (A), turnip (B) and apple (C) tissues having similar porosity (± 29\%) at big ROI (top) and the pore network with reduced ROI size (bottom).}
\end{figure}

Tissue of eggplant, turnip and apple having identical porosities (± 29\%) exhibited variability in local thickness and connectivity of the intercellular space network (Figure 3). At big ROI, it was clear that eggplant and turnip tissues were dominated by the small intercellular spaces while apple tissue was arranged by larger intercellular spaces. The number of the intercellular space was lower for eggplant (42.00) compared to turnip (74.00) and apple (79.00), implying more connected intercellular space in eggplant tissue. The lower the number, the more connected the intercellular spaces are. Continuous pore network throughout the tissue would have a value 1 [2]. Eggplant intercellular space also had more branching number (2234), followed by turnip (1271) and apple (141).

Pore tortuosity based on connected pores between two opposite sides of the tissue geometry highly varied depending on the local microstructure. High tortuosity increases the resistance to diffusion of oxygen through a porous plant tissue. The result implies that the plant tissues with similar porosities may have a large variance in effective O\textsubscript{2} diffusivities, depending on the characteristic of their pore structures as channels for respiratory gas transports, also explaining different O\textsubscript{2} diffusivity-porosity correlations for different plant products. The reduction of ROI size altered the microstructural parameter values of plant organ tissues (Table 1). This explains the change in the distribution pattern of the built correlations at small ROI. Although the R\textsuperscript{2} of the correlation increased, the small ROI that was situated in the center of the big ROI was still affected by complexity of pore structure. Determination of a computational domain size was critically essential where variability of a calculated gas diffusivity was high with reducing ROI size [4].
Table 1. Microstructural analysis of eggplant, turnip and apple tissues at two different ROI sizes.

| ROI size (mm³) | Product | Porosity, ε (%) | Tortuosity, τ | Number of pore | Branching number | Deff. O₂ (x10⁻⁶ m²/s) |
|---------------|---------|-----------------|--------------|---------------|------------------|---------------------|
| 1.1x1.1x1.1   | Eggplant| 30.63±1.37      | 15.55±15.88  | 42.00 ±7.00   | 2234±1433        | 1.40±0.84           |
|               | Turnip  | 29.13±0.56      | 4.16±1.14    | 74.00±27.00   | 1271±581         | 1.70±0.17           |
|               | Apple   | 29.10±0.55      | 10.31±5.54   | 79.00±18.00   | 141±69           | 0.24±0.08           |
| 0.2x0.2x0.2   | Eggplant| 29.97±5.33      | 2.32±2.32    | 4.00±3.00     | 30.00±24.0       | 1.82±1.35           |
|               | Turnip  | 17.27±3.71      | 5.52±3.59    | 5.00±2.00     | 18.00±21         | 0.69±0.54           |
|               | Apple   | 35.97±16.09     | 3.20±2.29    | 3.00±2.00     | 2.00±1.00        | 3.88±3.62           |

4. Conclusion
Effective O₂ diffusivities did not linearly correlate with porosity. The correlation was considerably affected by micro-architecture of intercellular space. The reduced ROI size minimized the complexity of the tissue intercellular space improving coefficient of determinant (R²) of the O₂ diffusivity-porosity relationship for all samples. However, effect of microstructural properties to the correlation was still existed. In a next step, the correlation with bigger ROI will be studied. It is expected that O₂ diffusivity is no longer influenced by the complexity of tissue microstructure so that effective O₂ diffusivity only links to the tissue porosity.

References
[1] Dražeta L, Lang A, Hall A J, Volz R K and Jameson P E 2014 Air volume measurement of 'Braeburn' apple fruit Journal of Experimental Botany 55: 1061-1069.
[2] Herremans E, Verboven P, Verlinden B E, Cantre D, Abera M, Wevers M and Nicolaï B M 2015 Automatic analysis of the 3-D microstructure of fruit parenchyma tissue using X-ray micro-CT explains differences in aeration BMC Plant Biology 15:264.
[3] Nugraha B, Verboven P, Janssen S, Wang Z and Nicolaï B M 2019 Non-destructive porosity mapping of fruit and vegetables using X-ray CT Postharvest Biol. Technol. 150: 80–88.
[4] Ho Q T, Verboven P, Verlinden B E, Herremans E, Wevers M, Carmeliet J and Nicolaï B M 2011 A three-dimensional multiscale model for gas exchange Plant Physiol. 155: 1158–1168.
[5] Herremans E, Verboven P, Defraeye T, Rogge S, Tri Q, Hertog MLATM, Verlinden B E, Bongaers E, Wavers M and Nicolaï B M 2014 X-ray CT for quantitative food microstructure engineering: The apple case. Nucl. Inst. Methods Phys. Res. B 324: 88–94.
[6] Al-raoush R I and Madhoun I T 2017 TORT3D : A MATLAB code to compute geometric tortuosity from 3D images of unconsolidated porous media Powder Technology 320: 99–107.
[7] Verboven P, Kerckhofs G, Mebatsion H K, Ho Q T, Temst K, Wevers M, Cloetens P and Nicolaï B M 2008 Three-dimensional gas exchange pathways in pome fruit characterized by synchrotron X-ray computed tomography Plant Physiol. 147: 518–527.