Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

Modeling Chlorophyll Fluorescence of Kiwi fruit (*Actinidia deliciosa).*

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Supplementary information

Multireflections for the excitation fluxes

Between the pulp and the peel multiple light reflections can take place. These reflections for the excitation beams are shown schematically in Figure 1.

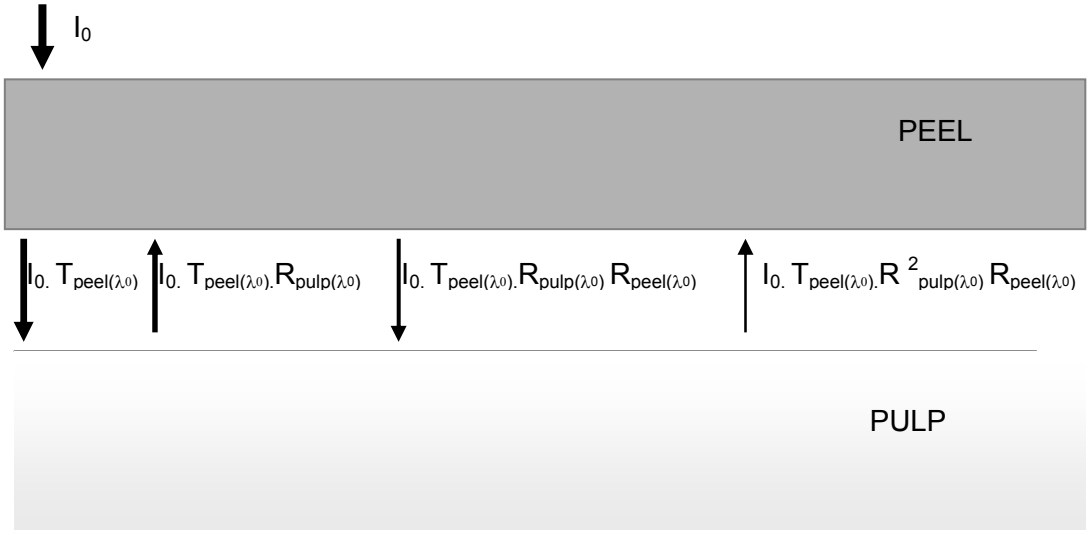

Figure 1. Multireflections for the excitation fluxes between the peel and the pulp

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The fluorescence generated inside the pulp (I_f) is given by

$$I_{f(\text{pulp in the fruit})} = I_a \cdot \Phi_f \qquad (1)$$

Where I_a is the light intensity absorbed by the pulp and Φ_f is the fluorescence quantum yield for the pulp. Actually I_a has several contributions due to the multiple reflections of the excitation fluxes. When a quantity $I_0(\lambda_0) \cdot T_{peel(\lambda_0)}$ (I_0: intensity of the excitation beam, λ_0: excitation wavlength, T_{peel}: peel transmitannce) reaches the pulp, a quantity $I_0(\lambda_0) \cdot T_{peel(\lambda_0)} R_{pulp\lambda_0}$ (R: reflectance) is reflected and a portion $I_0(\lambda_0) \cdot T_{peel(\lambda_0)} (1 - R_{pulp\lambda_0})$ is absorbed (the whole pulp is optically thick and the transmittance is approximately zero). As the reflected ray is reflected again at the peel a quantity $I_0(\lambda_0) \cdot T_{peel(\lambda_0)} R_{pulp\lambda_0} R_{peel\lambda_0}$ comes back to the pulp. As a consequence, another portion of light is absorbed by the pulp: $I_0(\lambda_0) \cdot T_{peel(\lambda_0)} R_{pulp\lambda_0} R_{peel\lambda_0} (1 - R_{pulp\lambda_0})$ and the multireflection process continues in this way.

By adding up the infinite contributions to the absorbed light, it can be deduced that:

$$I_a = I_0(\lambda_0) \cdot T_{peel(\lambda_0)} (1 - R_{pulp\lambda_0}).(1 + r + r^2 + r^3 + \ldots + r^n) \qquad (2)$$

where $r = R_{pulp\lambda_0} R_{peel\lambda_0}$

Taking into account that r <1, equation (2) is reduced to equation (3).

$$I_a = I_0(\lambda_0) \cdot T_{peel(\lambda_0)} (1 - R_{pulp\lambda_0}).\frac{1}{1 - r} \qquad (3)$$

In our experiments, at the excitation wavelength 460 nm, **r** value was lower than 0.005 and for excitation at 594 nm, it was lower than 0.04. Then, in the physical approach presented in the manuscript we have neglected the contribution of the multiple reflections for the excitation flux to the light absorbed by the pulp.

Multireflections for the fluorescence flux

The fluorescence emission generated in the pulp can also present multiple reflections at the boundary pulp-peel. In Figure 2, a picture schematizing the situation is shown.

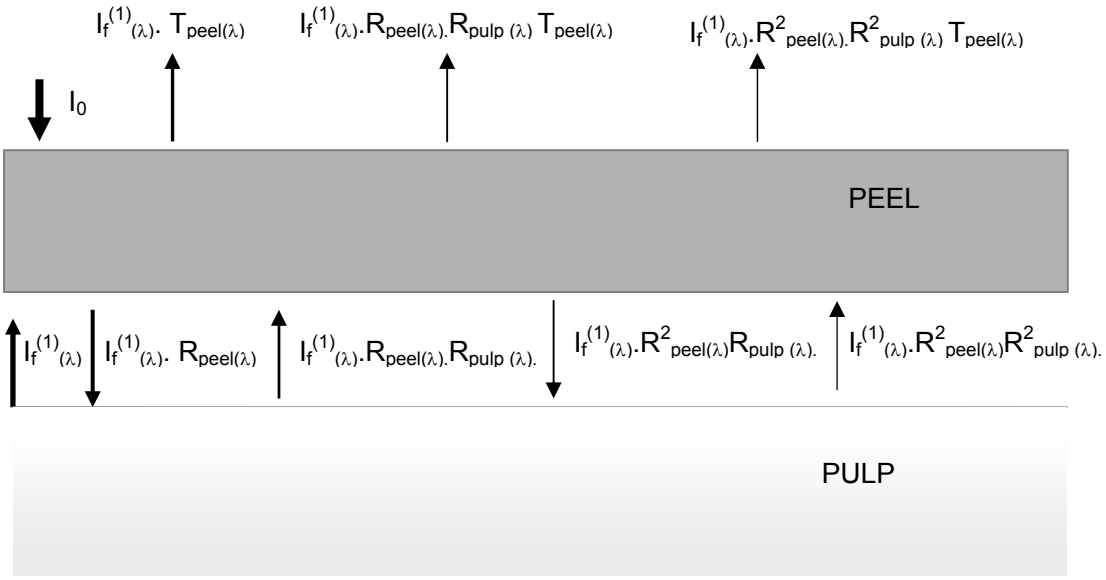

Figure 2. Multireflections for the fluorescence fluxes between the peel and the pulp

In Figure 2, $I_f^{(1)} = I_0(\lambda_0) \cdot T_{peel(\lambda_0)}(1 - R_{pulp\lambda_0}).\Phi_f$. In this scheme, the fluorescence that might be generated in the pulp from the "reflected emission" was considered completely negligible compared to that generated from the incident beam. Under these assumptions, the emission from the pulp obtained out of the fruit may be written as:

$$I_f = I_f^{(1)}T_{peel(\lambda)}. + I_f^{(1)}.R_{peel(\lambda)}.R_{pulp(\lambda)}T_{peel(\lambda)} + I_f^{(1)}.R^2_{peel(\lambda)}.R^2_{pulp(\lambda)}T_{peel(\lambda)} + \ldots \qquad (4)$$

where λ is the emission wavelength.

Equation (4) may be re-written as:

$$I_f = I_f^{(1)} T_{peel(\lambda)} (1 + r + r^2 + r^3 + ... + r^n) \quad (5)$$

where $r = R_{peel(\lambda)} R_{pulp(\lambda)}$

Again, taking into account that r <1, equation (5) is reduced to equation (6).

$$I_f = I_f^{(1)} T_{peel(\lambda)} \frac{1}{1-r} \quad (6)$$

So, multiple reflections of fluorescence at the boundary peel-pulp would affect the observed fluorescence I_f at each wavelength by a factor 1/(1-r) that is different in this case for each emission wavelength. However, considering the most adverse situations (the highest product of reflectances) this factor introduces an error lower than 10% in $I_f(\lambda)$ and it was neglected in the theoretical approach presented in our manuscript.

Validation of the correction model for the pulp

To validate the correction model that accounts for light re-absorption processes in the case of the pulp, the following extra experiment has been performed.

A portion of the pulp was deposited on the quartz plate and it was gently crushed by means of a glass rod. Then the material was spread on the plate using a sharp blade so that a thin film layer of pulp was obtained. The sample was dark adapted during fifteen minutes and fluorescence spectra were recorded under low photon flux (as stated in the manuscript in the section Materials and Methods. Original Fluorescence). Several spectra were obtained with decreasing thickness of the pulp layer until no variation was found in the experimental fluorescence ratio. This spectrum, which was assumed to be free of light re-absorption, is shown in Figure 3.

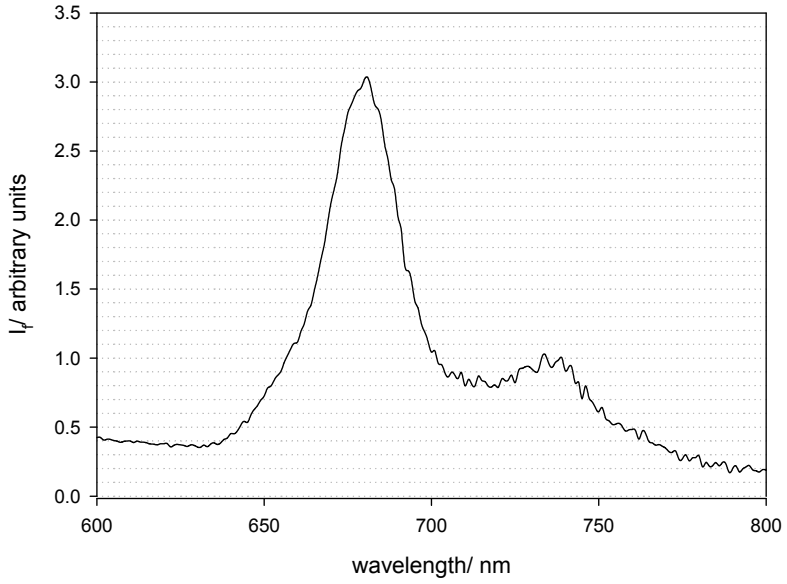

Figure 3. Fluorescence spectra corrected for the detector response for a thin layer of pulp where no light re-absorption is present. Excitation wavelength: 460 nm.

The spectrum presented in Figure 3 was the result of the average from five samples of thin layers of pulp. The value of the fluorescence ratio obtained from these samples resulted to be 3.0 ± 0.4. This value agrees with the fluorescence ratio obtained in our work after correction of the experimental pulp to account for light reabsorption (see Figure 4 and Table 1 in the manuscript).