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Fluorimetric Analyses of Fruit Pigments

Factors Disturbing the Fluorescence Signal

Reflectance spectroscopy has been established as an analytical method of assessing fruit ripeness and quality with handheld units or in sorting lines. With the higher sensitivity of fluorescence spectroscopy, it is also possible to measure individual fruit compounds, which only appear in extremely low quantities. However, its application in practice is not possible till now, because the reabsorption and fluorescence quenching effects in the complex fruit tissue influence the qualitative and quantitative analysis of the fluorescence signal.

Non-destructive methods to improve process monitoring became more and more important in horticulture. Optical methods such as reflectance spectroscopy have shown to be an adequate tool to evaluate quantitatively changes of fruit and vegetable compound contents during ripening on the plant and post-harvest [1]. They were already used as hand-held systems or are integrated in sorting lines as fast and non-destructive methods to measure fruit and vegetable internal quality during production and processing.

Organic and inorganic molecules are able to absorb light, but only a part of them posses the ability to re-emit the absorbed energy as fluorescence [2]. Therefore the fluorimetric detection is more selective than the photometric determination of absorbance and reflectance attributes. Moreover, due to the higher sensitivity, also small amounts of fluorescing compounds can be detected with the help of fluorescence spectroscopy. In fruits and vegetables some of the health-promoting secondary plant compounds (vitamins, phenols) are native fluorophores. In contrast, the distinctive chlorophyll fluorescence analysis is used already, particularly, for the detection of the leaf photosynthetic activity in the field.

The quantitative analysis of chlorophyll as well as minor contents of single compounds in the fruit tissue, based on their fluorescence emission, is affected by exogenous parameters and inter- and intra- molecular interactions. In the present study the reabsorption und quenching effects on the fluorescence signal have been investigated in standard solutions and on minimally processed fruit tissue. The fluorescence emission spectra were recorded on a spectrophotometer (Prototype, SAFAS S.A., Monaco) in front-face mode using a xenon-flash-lamp and a photomultiplier for the signal detection in a wavelength range from 200 to 700 nm. For the data processing, the fluorescence spectra were corrected with simultaneously recorded reflectance spectra, as commonly applied in practice.

Complexity of influencing factors

Fruits and vegetables contain several autofluorescent molecules such as pigments (e.g. chlorophyll), vitamins (e.g. riboflavin, tocopherol), NADPH, and phenolic substances (e.g. derivates of cinnamic acids) [3]. The composition and contents of both, fluorescent and non-fluorescent fruit compounds, are determined by the biotic and abiotic factors during processing and post-harvest conditions (*Fig. 1*).

Moreover, interactions between the fluorescing molecule and the molecules` environment in the fruit tissue and filter effects lead to variances of the fluorescence excita-

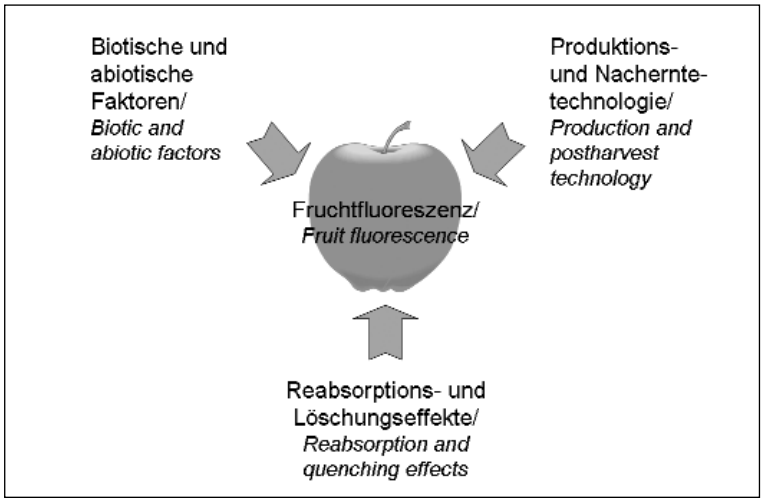

Fig. 1: Complexity of the factors influencing the fluorescence signal emitted from the fruit matrix

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tion and emission characteristics. When fluorescence quenching effects occur, the intensity of the wavelengths specific fluorescence does not reflect the actual content of the fluorophore; the quencher reduces the fluorescence intensity. Then again, the effective fluorescence signal of a single molecule in solution or in the fruit tissue can also be reduced by its own with increasing concentration. For this reason, the fluorescence quenching caused by high concentrations of the fluorophore has been tested in solutions of the relevant fruit compounds (single phenols). For the natively occurring concentrations of the compounds studied in the fruit tissue, the fluorescence intensity was linear with the concentration. From this point of view, the fluorescence spectroscopy can therefore be used to non-destructively detect the natural contents of secondary plant compounds in fruits.

However, in a complex matrix such as fruit tissues, competing filter effects of the light absorbing molecules have to be taken into account when measuring fluorescence spectra. The intensity of the fluorescence emission is proportional to the excitation light. When other molecules absorb the light energy, the fluorescence intensity will be reduced. Moreover, emitted photons can also be absorbed by other molecules before the fluorescence signal could be detected.

The influence of these reabsorption and quenching effects can be seen for instance during browning reactions in sliced apples. In the present study, the fluorescence emission spectra were measured on the fruit tissue directly after cutting and during 60 minutes (*Fig. 2*) in 2-minutes interval. The fluorescence intensity in the blue-green wavelength range (400 to 550 nm) decreased due to the reduction of single phenols. At the same time the reflectance intensity was also decreasing as a result of the absorption of the newly built polyphenols, which were responsible for the brown colour of the sliced fruit tissue, and which reabsorb the fluorescence of the nutritional valuable phenols. Commonly, the real fluorescence emission is calculated when correcting the apparent fluorescence signal at each wavelength by dividing it with the reflectance intensity. The corrected fluorescence showed only a slight decrease of the phenol content compared to the apparent fluorescence.

In the red wavelength range (around 680 nm) the chlorophyll fluorescence was measured, which decreased after cutting due to oxidative reactions. The correction of the fluorescence signal with the reflectance intensity indicated an underestimated reduction of the chlorophyll fluorescence. The newly formed polyphenols compete with the chlorophylls for the excitation light. Hence, the apparent fluorescence emission was proportionally reduced. The distinction between these two phenomena was possible by regarding the relative fluorescence signal related to the simultaneously recorded fruit reflectance spectra.

The exemplarily presented influencing effects on the fluorescence signal detection, which were accelerated during the browning reactions, occur also in the complex fruit matrix and complicate the analysis of the measured apparent fluorescence signal.

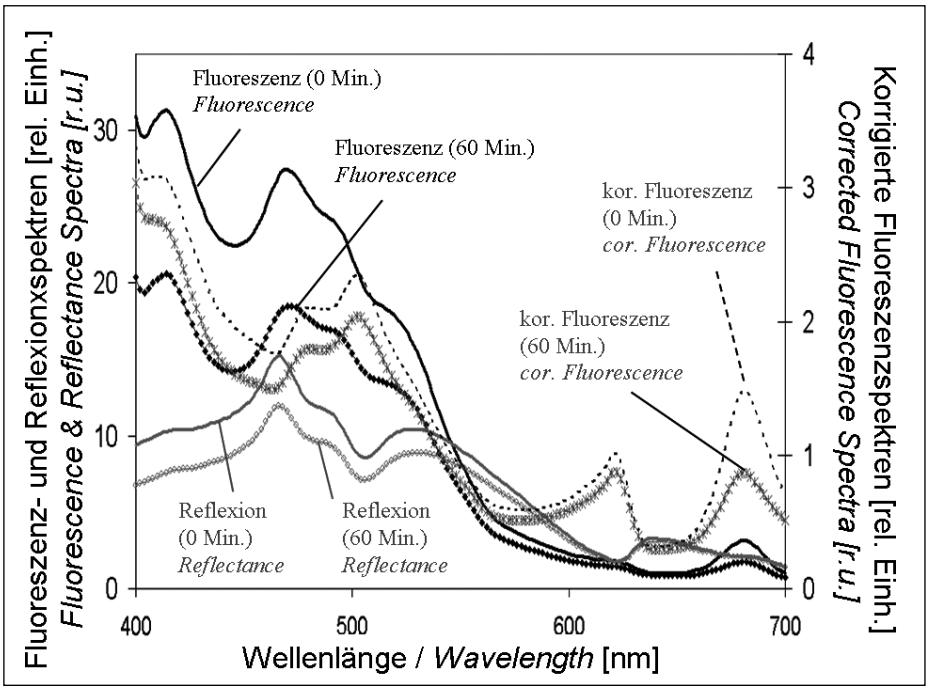

Fig. 2: Fruit fluorescence spectra, diffuse reflectance spectra, and relative fluorescence spectra measured on the fruit tissue, directly after cutting the apple (0 minutes) and 60 minutes later

Outlook

The determination of secondary plant compounds and health-promoting substances in horticultural products using fluorescence spectroscopy is hindered due to the complexity of the fruit matrix. However, the sensitivity of the measuring method was adequate to detect the natural occurring compound contents. With the help of simultaneously recorded reflectance spectra or with advanced multivariate data processing methods it could be possible in the future to take the reabsorption and quenching effects into account for a qualitative and quantitative analysis of the fluorescence signal. Then, fluorescence spectroscopy can be effectively used for the optimisation of fruits and vegetables processing with regard to their nutritional value.

Literature

Books are marked by •

[1] *Zude, M.*: Innovation: Mit optischer Sensorik zerstörungsfrei die Fruchtreife und -qualität entlang der Versorgungskette bestimmen. Fruchthandelsmagazin 6 (2005), S. 44-45

[2] • *Guilbault, G. G.*: General Aspects of luminescence spectroscopy. In: Guilbault, G. G. (Hrsg.): Practical Fluorescence. 2. Auflage. Dekker-Verlag, New York, 1990, pp. 1-40

[3] *Buschmann, C., G. Langsdorf and H.K. Lichtenthaler*: Imaging of the blue, green and red fluorescence emission of plants: An overview. Photosynthetica 38 (2000), no. 4, pp. 483-491