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Chlorophyl fluorescence imaging analysis and fluorescence spectra analysis

Possible horticultural and agricultural applications

More cost-effective and flexibly-applicable measurement systems as well as rapid advances in laser technology along with improved measurement methods have in recent years extended the application potential of non-destructive fluorescence analysis in horticultural and agricultural research. This has meant the commercial availabilty of powerful imaging analysis systems in recent times and with this the possibilitiy of increasingly reliable results in product quality assessment, grading, variatal screening and plant breeding. Here, the fundementals are discussed along with a number of problems still remaining in the application.

Currently, comprehensive quality control from production to consumer is encouraged throughout agriculture and horticulture. An objective quality description is only achievable through a modern, product-oriented quality definition based on chemical and physiological product characteristics. Here, the requirements for precision and consistency of applicable parameters and, above all, the methods used, are high. Advances in electronics, data processing technology and natural sciences accelerate the developments of new, non-destructive, sampling methods and systems which are currently being tested in practice [1].

One can assume that with fresh products only those with high (potential or actual) metabolic activity also have a high (inner) quality. In green plant tissue photosynthesis is an important physiological process which links many biochemical and biophysical reactions and is influenced from internal and external stimulations in multiple ways. This makes the determination of photosynthetic activity, and its reactions to different stresses, into a potent tool for the evaluation of product quality. The chlorophyl fluorescence analysis delivers non-destrcutive rapid and/or complex information on performance capabilities and integrity of photosynthesis [2, 3]. Easy to operate fluorescence analysis measurement equipment [2] as well as application of modern imaging analysis technology mean such methods are versatile indicators of physiological activity within chlorophyl content plants and plant parts and have aided their distribution in horticulture research [3, 4].

Chlorophyl fluorescence analysis

In the chloroplasts of green plant tissue during „undisturbed“ photosynthesis light energy is absorbed by chlorophyls and primarily converted into biochemically-usable energy (photochemistry). This is mainly used for the synthesis of sugars from carbon dioxide and water. A small proportion of the absorbed light is given-off by the photosynthesis pigments as heat and around 2 to 3% further as fluoresence.

If this photochemical energy utilisation is disturbed, slowed or completely stopped in a stress situation (heat, cold, frost, drought, lack of oxygen, pathogenic infection, herbicide application....) whilst radiation remains unreduced, there occurs an „energy jam“. This induces a series of protection mechanisms which leads to an increased conversion into heat and finally also to increased fluoresence [2].

Modern fluorometers [3] record, however, more than the continuous fluorescence. They also win information regarding activity and

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Keywords

Chlorophyllfluorescence analysis, fluorescence spectra analysis, imaging analysis, photosynthesis, product quality

Literature

Literature details are available from the publishers under LT 02215 or via Internet at http://www.landwirtschaftsverlag.com/landtech/local/fliteratur.htm

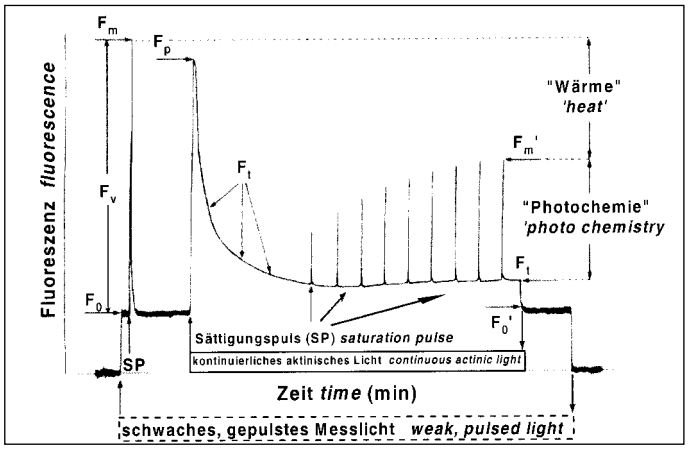

Fig. 1: Example of a typical fluorescence transient as recorded with a PAM-fluorometer.

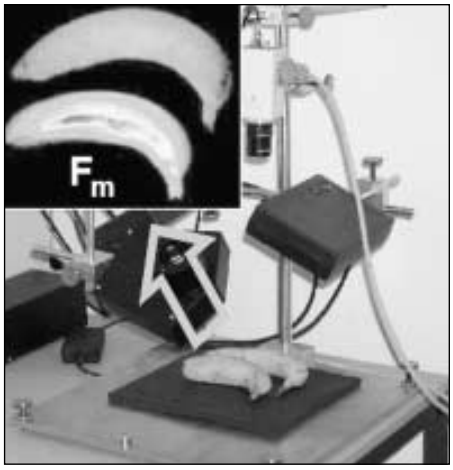

Fig. 2: The chlorophyll fluorescence imaging system FluorCam (Photon Systems Instruments) consists oiLED-panels, habogene saturation-pubs lamp, CCV camera and control unit. The very small green parts of the partially ripe banana are easily visible in the lower chlorophyll fluorescence image.

integrity of the photosynthesis apparatus and the relative activity of the protection mechanisms. A suitable measuring protocol is necessary depending on the parameter to be determined.

For a comprehensive analysis (*fig. 1*) the product is darkened for a few minutes. This „switches off" heat emission and puts photochemistry into „stand by". Illumination then with a weak nonphotosynthetic-active measurement light induces so-called basic fluorescence (F_0) without development of any photochemical results. A short light impulse of very high intensity (saturation pulse) activates all chlorophyl molecules short-term. During this time neither photochemistry nor heat conversion is active so only the fluorescence remains to emit the absorbed energy. This maximises the fluorescence (F_m). Subsequent continuous illumination reflects the fine regulation of the photosynthesis through characteristic alterations in the fluorescence from F_0 to F_P through to steady state signal (F_t). After reaching F_t saturation light impulse induces only an intermediate maximum (F_m') because the heat radiation is active through the continual illumination. On the other hand, the rise to F_m' shows that a part of the possible fluorescence is normally stopped through the photochemical results. Through appropriate calculation of the fluorescence signal one can evaluate the relative proportoin of the photchemistry as well as the heat on the utilisation of the absorbed light energy.

While a complete fluoresence analasys lasts about 30 mins. (*fig. 1*), one achieves through the measurement of F_t and F_m' valuable results rapidly. In this way the quotient (F_m'-F_t)/F_m' is a measurement for the actual photochemical efficiency. If one also measures the absorbed amount of photons one can determine the electron transport rate from the quotient. The relationship of the variables (F_m -F_0) to the maximum fluorescence F_m, on the other hand, describes the maximum photochemical efficiency. In that only an intact system can be maximally efficient, this enables statements on the integrity of the photosynthesis apparatus.

However there is often a well-developed spacial dynamic to be found in the stress reaction [4]. Ignoring this effect increases the variability of the results and can lead to mistaken interpretations. The imaging analysis [6] and the time-resolved spectra analysis [7] offer reliable application in such cases.

Chlorophyl imaging analysis

Various self-built systems have already been applied with succcess [5]. For some time now commercial chlorophyl imaging analysis systems have been available [4]. With this, nearly all the problems mentioned so far can be investigated with relatively high spacial and timely resolution.

Even very low chlorophyl contents can be sensitively identified through fluorescence analysis as emphasised in the comparison between two bananas in *figure 2*. The completely ripened and discoloured fruit gives only a weak consistent fluorescence signal. With the second fruit, also showing only a very small, hardly discernable green pigment proportion, a clear chlorophyl fluorescence (F_m) locality in some parts of the fruit can be determined. Thus this system is useful, for instance in grading or recognition of ripeness stage [6].

Laser-induced fluorescence spectra analysis (LIFS)

Healthy apples were mechanically stressed in the laboratory until the fruit tissue disintegrated and with the help of of LIFS the appearance of a dark pressure point after 40, 60, 135 and 180 minutes determined. For this radiation from an Argon ionen laser was applied in the wavelength range 351 to 364 nm. The chlorophyl thus stimulated emitted a radiation in the dark red wavelength range whilst also stimulated phenolic compounds emitted in the blue-green wavelength range. Alterations in the chlorophyl and polyphenol content through development of the pressure points could be measured though change in the fluorescence intensity the specific wavelengths. Multivariate evaluation methods are required for interpretation of the results because the light emissions from different molecule groups are simultaneously recorded [7]. The evaluation of the fluorescence spectra shows a clustering caused through the increase in the fluorescent polyphenols in the tissues during the development of the visible pressure points in the process of the recording (*fig. 3*). Fluorescent spectra in the blue-green wavelength range can in this way be applied for non-invasive investigation of physiological processes and also for non-destructive quality controls [8]. In this particular case there remains, however, substantial research requirement.

Summary

Chlorophyl fluorescence analysis and fluorescence spectra analysis offer outstanding, when not completely problem free, possibilities for investigation of quality or stress reactions in horticultural products. For a comprehensive quality analysis the selection of the correct measuring parameters based on the results required is important. Biotic and abiotic factors can, when their effects are not sufficiently taken account of, influence both result and its interpretation.

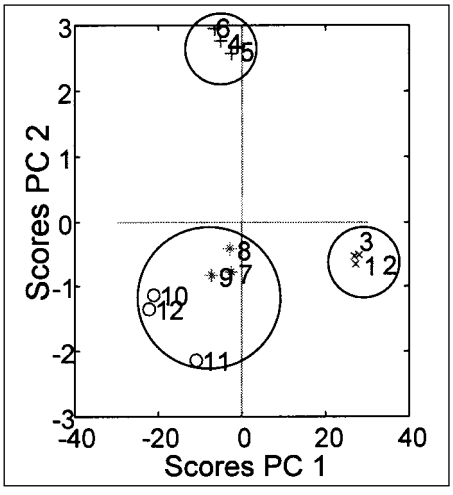

Fig. 3: The PCA of a LIFS (400-580 nm) of mechanical loaded apples (n = 3) during the formation of a brusing damage (1-3= 40, 4-6= 60, 7-9= 135, 10-12= 180 min after impact)