Laser-Induced Fluorescence Spectroscopy (LIFS) - a Non-Destructive Method to Detect Tissue Browning

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Keywords: apple; banana; tissue browning; fruit salad; fluorescence

Abstract

Laser-induced fluorescence spectroscopy (LIFS) was non-destructively applied on sound and bruised apples. Furthermore, the technique was tested to measure surface browning of freshly cut banana slices, which were treated with different anti-oxidative additives.

Changes in the apple fruit composition after bruising become visible in intensity variations of the blue-green and red fluorescence. Tissue browning of banana slices was detected by changes in the blue-green fluorescence. Such effects were obtained before the symptoms could be visually noticed. Results of the present study point out the potential of LIFS to non-destructively detect physiological changes of fruit composition during the entire processing chain of fresh fruit.

INTRODUCTION

During recent years, there has been an economical need for high quality horticultural products. At the same period consumer's preference for convenience has largely increased. The food industry is responding with an increased range of products and now, we are finding more fresh sliced salads (ready-to-eat salads) in the retail trade than ever. It appears that the sliced products are spoiling more quickly, and resulting there is a higher uncertainty of quality and hygiene, which is requiring more monitoring and controls to be put in place. This results in stricter legal constraints and more stringent quality management on the producer's side.

Consequently, to meet these requirements, various technologies were applied to ameliorate the quality monitoring. However, brown discolouration often occurs on the surface of the fresh or sliced fruit, a severely negative aesthetic trait, resulting in a decreasing demand from the consumer. Suitable additives are used up until now to reduce the loss in aesthetic value and to avoid tissue decay. Such additives have been based on acids as ascorbine and benzoe, which considerably affects the flavour of the product. Such diminutions can be avoided by optimising these supplements for fresh convenient products in terms of concentration and composition.

In this field of research, the optical technology belongs to the most innovative and most important non-destructive measuring methods. Therefore, controlling and monitoring the quality in the entire processing chain of fresh fruit represent a potential field of application for the fluorescence spectroscopy.

Recent research in fluorescence spectral analysis has been focussed on changes in fruit chlorophyll content detectable as red fluorescence in the visible wavelength range (Kautsky and Hirsch, 1931; Willert et al., 1995; Buschmann et al., 1986), whereas other wavelength ranges were hardly investigated. Lichtenthaler (Buschmann et al., 2000) and

co-workers have used a fluorescence imaging system obtaining non-invasively images of leaves to detect changes in the blue-green fluorescence.

In the present work it was assumed that the laser induced fluorescence spectroscopy (LIFS) can be used to detect changes in fruit compound contents. Such method can be applicable for quality monitoring in the entire processing chain and even for producing and monitoring convenience products.

MATERIALS AND METHODS

The fluorescence spectrometer (Laser Fluoroscope LF301 Lambda, I.O.M., Germany) used is equipped with a fibre-optic probe, which allows recording the spectra directly from the sample surface (e.g. sound apple, banana slice). A nitrogen laser emits short, intense laser pulses at 337 nm. A coloured glass container placed between the laser and the focusing lens can change the excitation wavelength. In the case of installing the exchangeable glass container, the fluorescence radiation can be excited in an area of 380 nm to 620 nm. The fibre probe has a length of 3 m with a kernel diameter of 600 µm. This exciting as well as receiving fibre is coupled to a Y-coupler with a length of 0.50 m. The top of the fibre-optic probe is pasted into a high-grade steel tube shaped with an angle of 8° to avoid measuring diffuse reflectance. The acousto-optic tuneable filter (AOTF) makes it possible to vary the detection wavelength in 1 nm intervals. The signal is detected by a photomultiplier tube (350 nm – 820 nm) and subsequently processed with a high time resolution (100 ps gate width).

Laser-induced fluorescence was applied non-destructively (Fig. 1) on sound and bruised apple fruit, which were stored under controlled atmosphere conditions. Measurements were carried out during the storage. Measurements were carried out using time-gate positions of 6.5 ns and 4.5 ns and excitation wavelengths of 337 nm and 488 nm, respectively. In a second experiment the construction was used without the glass container measuring fresh banana fruit being peeled and sliced at 337 nm. The time gate position was set at 7.5 ns using an evaluation by means of a $\lambda\tau$ curve measurement. The optimal measuring position at the fruit surface was specified by readings of fluorescence spectra along the radius of the banana slice. Beginning with a start measurement without any additives, the banana slices were subsequently sprayed with distilled water, natural lemon juice and ascorbic acid solution, the last two ones with different concentrations. Measurements were repeated several times during 2 hours. The vitamin c concentration of spray solutions was estimated using a rapid colour test-set (Merck, Germany).

LIFS data set was subjected to the second derivative and integral calculus by Savitzky-Golay (Savitzky and Golay, 1964) using 70% smoothing. This calculation was performed with TABLE CURVE 2D (SPSS Science, USA).

RESULTS AND DISCUSSION

During the storage, bruising marks appeared visibly on the apple fruits. Measuring the fruit with a chlorophyll fluorescence imaging system (FluorCam, Photon Systems Instruments) shows the chlorophyll allocation after bruising and storage (Herppich, 2002) (Fig. 2). The storage led to chlorophyll breakdown and fluorescence spectra displacement visible on the red wavelength area. Furthermore, when comparing fluorescence spectra of sound and bruised apples using LIFS, changes in compound contents appeared (Fig. 3). This can be explained by the fact that bruising led to rapid chlorophyll breakdown resulting in a decrease in chlorophyll fluorescence intensity (peak at approx. 650 nm).

The λτ curve measurements were made to determine the exact time gate position and could be recorded at different wavelengths in the range from 350 to 820 nm. Referring to the fact that every fluorescent element was characterized by a typical absorption and fluorescence wavelength area and a specific lifetime (Zude, 2003), the time gate position to detect the influence of tissue browning on banana slices was determined at 7.5 ns (Fig. 4). Moreover, at the beginning of the second experiment cycle, measurements were made along the radius of a banana slice to detect the optimal measuring point (Fig. 5).

Fluorescence spectra varied over the profile of the slice as shown in Fig. 4 and 5. Regarding the intensity of these spectra (Fig. 6), the following measurements were recorded from the pale yellow and even part of banana fruit tissue with the least influence of peeling and dehydration effects from the border (point number 4).

The surface browning of banana fruit slices appeared within 2 hours after cutting. After spraying the fruit surface with distilled water, natural lemon juice (46 mg vitamin *c* L^{-1}) and ascorbic acid solution with lower (46 mg vitamin *c* L^{-1}) and higher (33 g vitamin *c* L^{-1}) concentration led to characteristic responses during tissue decay. Such physiological responses could be detected with the help of the fluorescence spectra using integral calculus (Fig. 7). Treating the slices with natural lemon juice and ascorbic solution at lower concentration resulted in only slight differences in changes of the fluorescence spectra. Otherwise, using vitamin *c* of higher concentration led to lesser fluorescence intensity breakdown compared to the start measurement, contrary to the aforementioned additives and to distilled water. Thus, it appears that LIFS could also be used to screen suitable additives for convenient products and their optimum concentration to avoid tissue browning.

CONCLUSIONS

The present study shows the potential of LIFS to determine changes in contents of fruit compounds affected by mechanical impacts like bruising or cutting. Such application would be beneficial for monitoring and controlling product quality in shelf life and during the entire processing chain.

Furthermore the laser induced fluorescence spectroscopy was used to determine tissue browning of banana slices being treated with different suitable additives. As a result, a potential application of the technique for rapid screening of anti-oxidatives in fresh fruit salads was pointed out.

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Figures

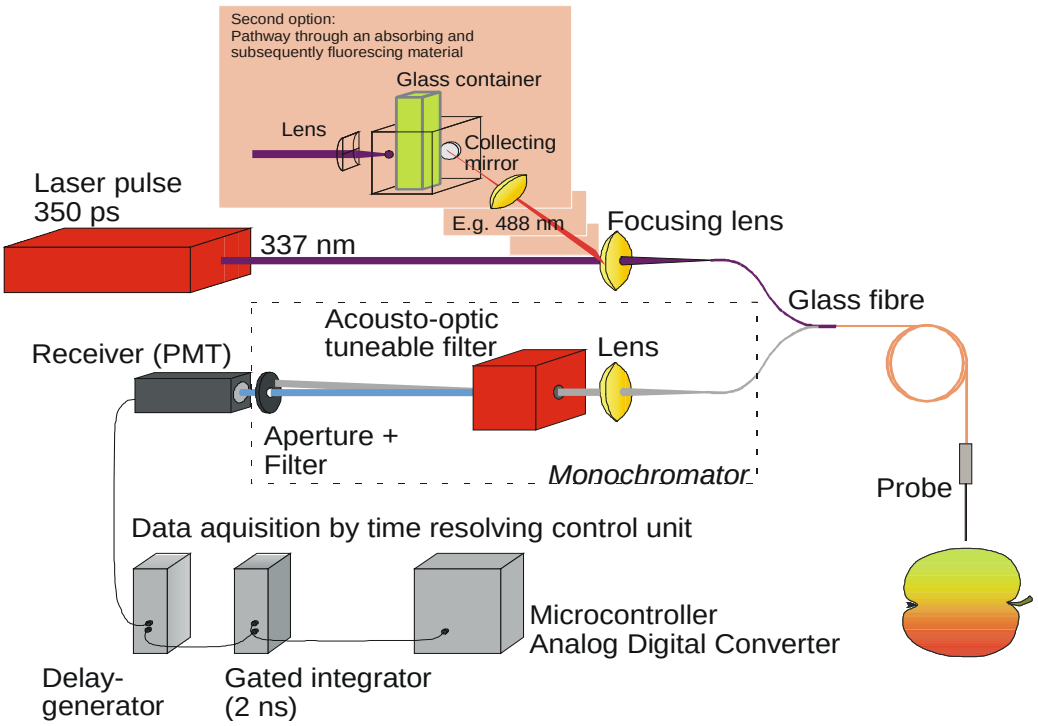

Fig. 1. Schematic view on the experimental set-up

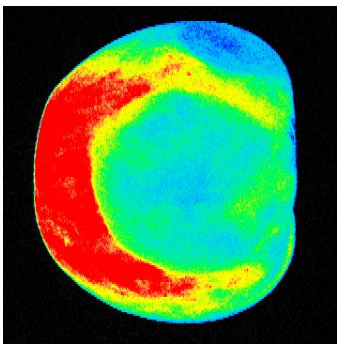

Fig. 2. Patchiness of chlorophyll of apple fruit visualized by changes in the F_M values.

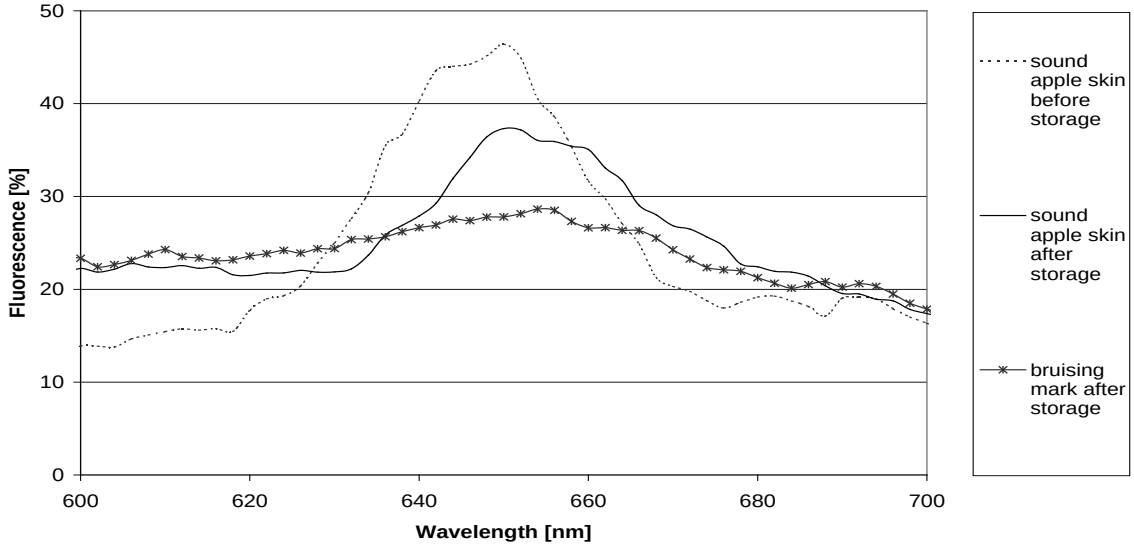

Fig. 3. Fluorescence spectra of sound and bruised apple skin.

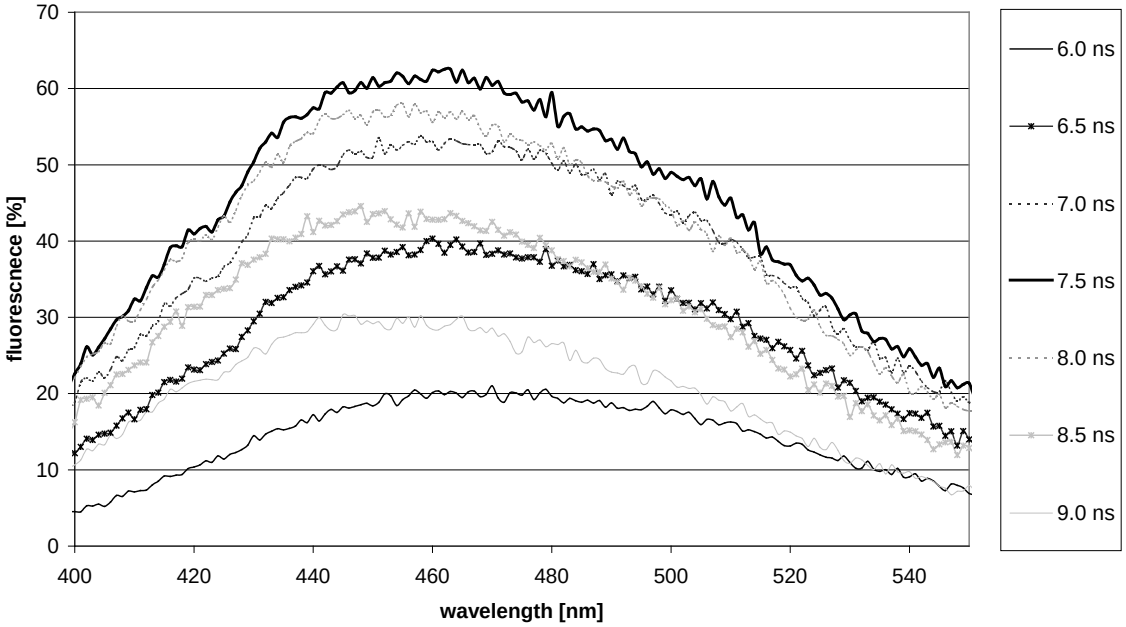

Fig. 4. The λτ curve measured on banana slice for determining the time gate position.

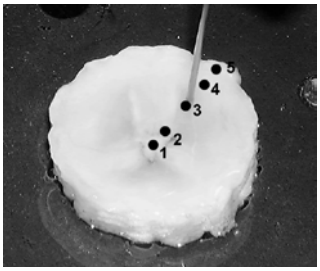

Fig. 5. Fluorescence spectra measurements at different measuring positions along the radius of banana slice.

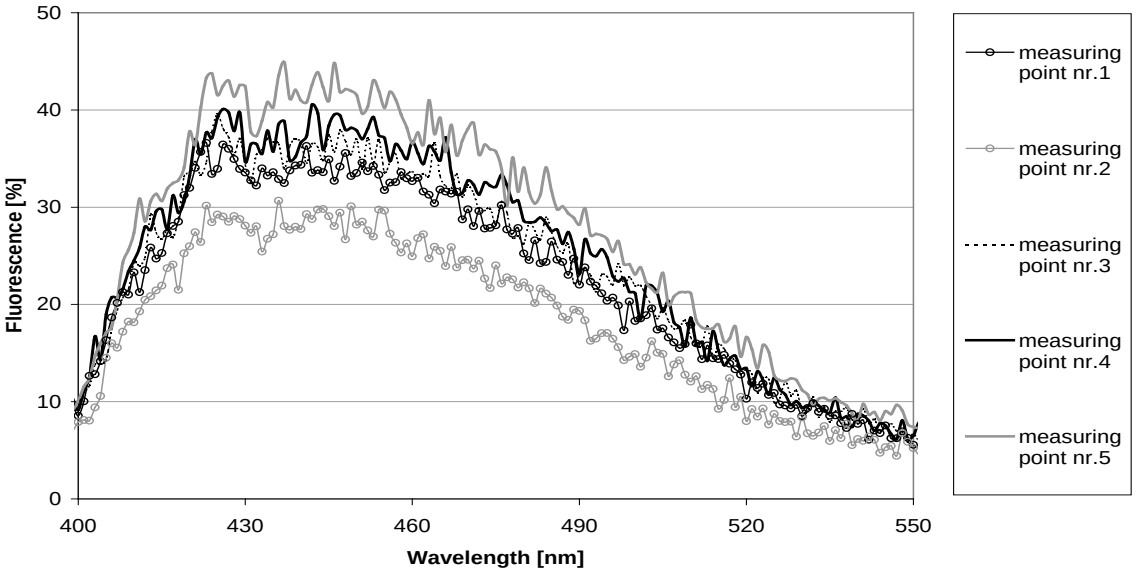

Fig. 6. Fluorescence spectra recorded at several measuring positions of banana slices.

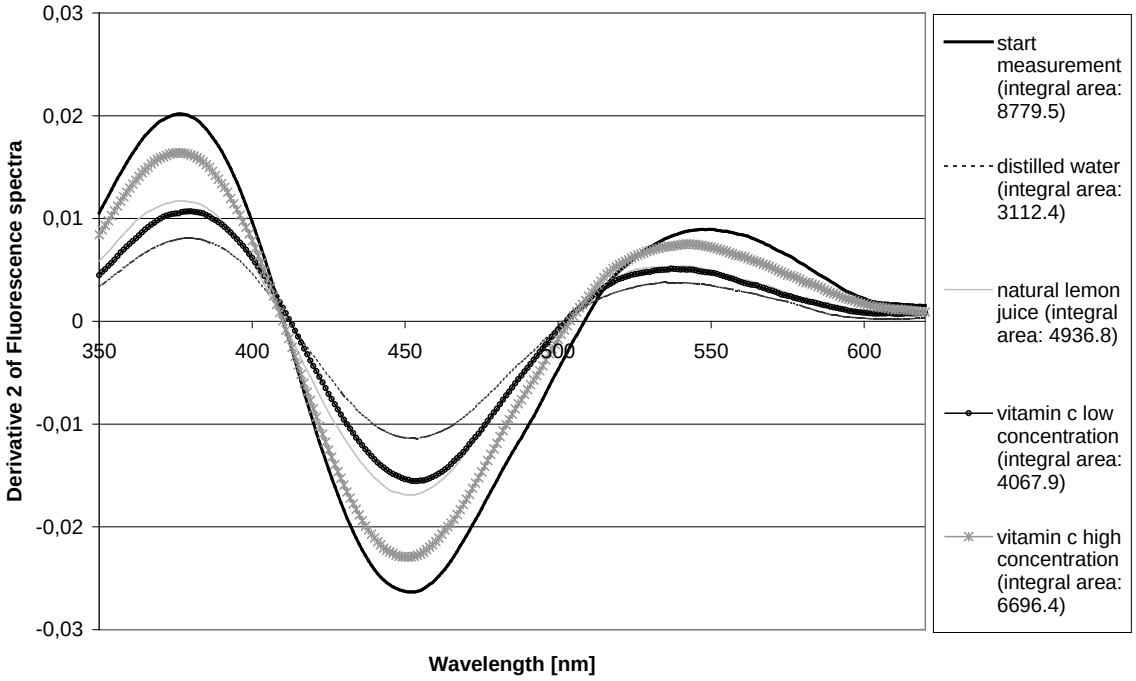

Fig. 7. Derivative 2 and integral min-max (Savitzky-Golay, 70% smoothing) of fluorescence spectra recorded at banana slices sprayed with different additives.