Studies of Gas Exchange in Fruits Using Laser Spectroscopic Techniques

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Abstract

Non-intrusive, *in-vivo*, real-time measurements of oxygen contents and oxygen diffusion in fruits using a laser spectroscopic technique are presented, where the narrow absorption features due to the free gas in pores in the fruits are observed. The technique is referred to as gas in scattering media absorption spectroscopy (GASMAS), and is performed using tunable diode lasers. In particular, assessment of oxygen transport in apples and oranges is demonstrated. To illustrate the possibility to use the technique for studies of modified atmosphere packaging processes, measurements on sealed horticultural produces were performed. Furthermore, preliminary studies of avocados concerning the possibility to non-intrusively determine the maturity of fruits are presented. This technique has substantial potential for the development of compact devices providing new types of information in postharvest fruit management.

INTRODUCTION

Assessment of free gas in porous materials is of considerable interest in several environmental, biological and medical processes. In the present paper a novel application of a laser spectroscopic technique to the study of gas exchange in porous agricultural products, in particular fruits, is discussed. Although any gas with absorption lines in reach of tunable diode laser sources can be probed by the technique, we here focus on biologically active molecular oxygen, whose concentration is of crucial importance for the ripening process and the quality of fruits. An overview of the influence of oxygen and carbon dioxide partial pressures on selected phenomena affecting fruit and vegetable quality has been given by, e.g., Beaudry (1999).

A common way to analyse gases *in situ* is to use absorption spectroscopy that employs a sufficiently narrow-band light source in combination with the Beer-Lambertian law. However, in porous materials the radiation is heavily scattered, which results in an enhanced average absorption path length compared to the geometrical dimensions of the sample. One approach to measure non-gaseous constituents in scattering media is to use time-resolved spectroscopy employing laser-produced white light; for a recent report, see Abrahamsson et al. (2005). This type of setup was recently used for studies on apples

(Cauchard et al., 2005). The spectral resolution needed in such systems is relatively low since solid materials and liquids have broad absorption features with linewidths normally not sharper than 10 nm, while free gases typically have a linewidth 10000 times sharper (Sigrist, 1994). Thus, the small and narrow absorption imprint in the emerging, multiply scattered diffuse light due to the gas can not be detected with such systems. However, by using single-mode continuous-wave lasers combined with modulation techniques the gas can be detected sensitively, without disturbance from the broader absorption features of the bulk material. In particular, diode lasers are highly suitable for sensitive absorption spectroscopy with high spectral resolution due to their availability in different wavelength ranges, uncomplicated wavelength tunability, and their relatively low cost compared to other laser sources. This methodology to study free gas embedded in scattering materials has been denoted GASMAS (gas in scattering media absorption spectroscopy) (Sjöholm et al., 2001; Somesfalean et al., 2002).

Measurements of molecular oxygen in wood have been performed in order to illustrate the influence of the density and anisotropy of the sample on the GASMAS signal (Alnis et al., 2003). Recently, studies of gas exchange in fruits, in particular apples, have been reported (Persson et al., 2005). The gas contents in the scattering medium gives rise to a signal of a certain strength, which is determined by the gas concentration as well as the average path length travelled by the light in the scattering medium. The latter quantity can be determined by time-resolved measurements allowing the true concentration to be determined (Somesfalean et al., 2002). In particular, transport of gas through the porous medium can be studied. Here, the sample is first exposed to a gas environmental anomaly; it may be placed for a few hours in a sealed plastic bag first flushed with pure nitrogen gas. Then, the sample is placed in the GASMAS setup and the time evolution of the atmospheric oxygen re-invasion is measured spectroscopically. Such gas transport in apples was first studied by Persson et al. (2005) in transillumination geometry, and is further investigated in the present paper in a more practical backscattering geometry. The technique presented may have powerful applications in the study of fruit physiology, controlled atmosphere (CA) storage and modified atmosphere packaging (MAP) performance.

EXPERIMENTAL PROCEDURE

Experimental arrangements for GASMAS measurements are shown in Figs 1 and 2a. The optics and electronics used are similar in the two arrangements and have been described more in detail by Persson et al. (2005). Two measurement geometries have been used; transillumination (Fig. 1) and backscattering (Fig. 2a). The spectroscopic light source used was a diode laser, Sharp LT031MDO, with a nominal output power of 7 mW. The wavelength of the diode laser was scanned by ramping the driving current at a repetition rate of 4 Hz across the R7R7 molecular oxygen line, which is situated at 761.003 nm (vacuum wavelength). Additionally, sinusoidal modulation of about 9 kHz was superimposed on the diode laser injection current to achieve a wavelength modulation of the light allowing sensitive wavelength modulation spectroscopy (WMS) using a lock-in amplifier. The laser radiation was guided to the sample by a 600 μm core diameter optical fibre.

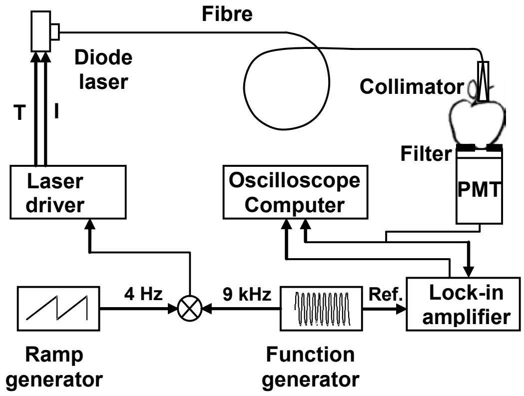

Fig. 1. Schematic layout of the GASMAS setup used for molecular oxygen detection in scattering porous materials such as fruits.

After light transport through the sample, a photomultiplier tube, Hamamatsu 5070A, detected the light. The ambient light was suppressed by a RG715 coloured glass filter used in combination with the sensitivity fall-off towards longer wavelengths for the photomultiplier tube. In the transmission geometry (Fig. 1) a circular aperture with a diameter of 6 mm was used to limit the detection area. The arrangement used in the backscattering geometry was slightly more complicated as can be seen in Fig. 2a. To launch the laser light into the scattering medium, a small right-angle prism providing total internal reflection was positioned in front of the fibre centrally located over the detector. The backscattered light that had travelled through the medium was collected in an annular aperture with an inner and an outer aperture diameter of 10 and 21 mm, respectively.

The DC and AC signals from the photomultiplier tube were detected separately. The DC signal, referred to as the direct signal, was sent to a digital oscilloscope, Tektronix TDS 520B. A high pass filter isolated the AC part and phase sensitive detection was performed with a lock-in amplifier. The component of the signal oscillating at the modulation frequency is similar to the first derivative of the direct signal, and, correspondingly, the component oscillating at twice the modulation frequency is similar to the 2nd derivative, etc. Since the direct signal is sloping, due to the simultaneous change in both wavelength and intensity as a function of injection current, the 1f signal is subject to a disadvantageous offset. This gives an advantage in using higher order components. However, the amplitude normally decreases by increasing the harmonic order. Thus, the component of the signal oscillating at twice the modulation frequency, i.e. 18 kHz, was selected as the output from the lock-in amplifier. This lock-in signal, referred to as the 2f signal, was sent to a second channel of the oscilloscope, which was computer controlled through GPIB communication by a LabVIEW program. An example of an oxygen signal recorded in backscattering geometry for the case of a 67-mm Granny Smith apple is shown in Fig. 2b.

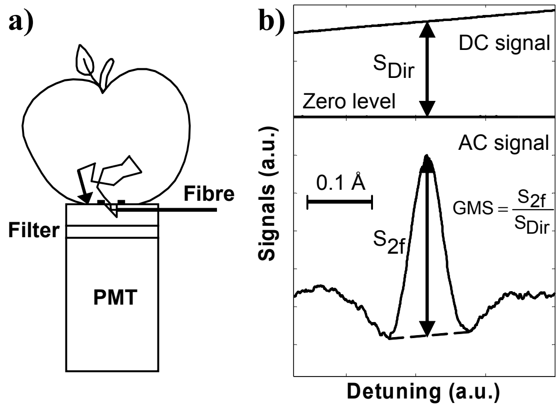

Fig. 2 (a). Modification of the GASMAS setup for backscattering (single-sided) measurements. (b). Direct absorption and second-harmonic component of the absorption for the R7R7 line in the oxygen A-band recorded from an Granny Smith apple in backscattering geometry.

As indicated in the figure the oxygen gas content can be evaluated from the peak-to-peak value of the absorption signature in the 2f signal. Clearly, the amplitude of the 2f signal is determined by the absolute size of the narrow gas absorption feature, i.e., to the fractional absorption due to the gas, and the amount of light reaching the detector. We are interested in the normalised GASMAS measurement signal, GMS, which is proportional to the fractional absorption. Thus, the AC signal (related to the oxygen content and the amount of light detected) was divided by the DC signal (related to the amount of light detected) as indicated in Fig. 2b. For small absorptions the GMS is proportional to the absorbance and thus to the product of the gas concentration and the path length travelled by the light. The standard addition method, just adding a path length of ambient air to be traversed by the laser light in addition to the scattering object under study, was adopted to calibrate a measured GMS and transform it into a more meaningful quantity. The acquired GMS values were plotted as a function of the added distance of air as discussed, e.g. by Persson et al (2005). The data points are expected to fall on a straight line, the zero intercept of which yields the equivalent distance, L_{eq}, in ambient air giving rise to a signal with the same magnitude as signal from the sample.

RESULTS AND DISCUSSION

Before presenting our new data, recorded mostly using backscattering geometry, we would like, in Fig. 3, to review some pertinent data obtained in our recent study (Persson et al., 2005). The recordings were performed in transillumination on Granny Smith apples using a setup of the type shown in Fig. 1. The gas content inside an intact fruit and the exchange of the gas was followed in real-time.

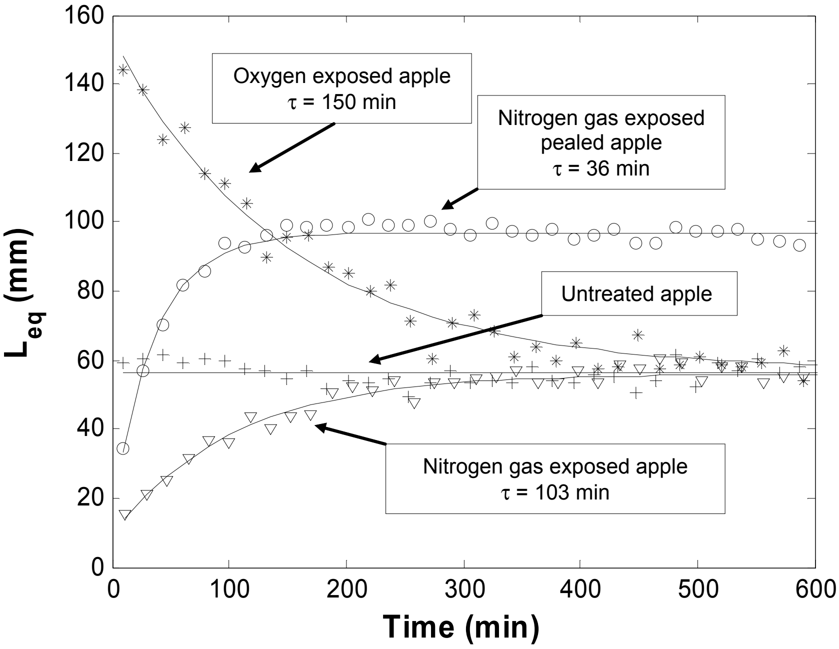

Fig. 3. Measurements of the gas exchange through differently treated Granny Smith apples in ambient air; untreated intact apple (+), intact apple pre-treated by immersion in nitrogen gas for 24 hours (□), pealed apple immersed in nitrogen gas for 12 hours (O) and another apple pre-exposed to an atmosphere with higher concentration of oxygen than in ambient air for 12 hours (). Exponential functions fitted to the data together with the estimated time constants are also included (Persson et al., 2005).

The equivalent mean path length, L_{eq}, was measured as a function of time for differently treated apples. A "flat" recording for an untreated apple, with a thickness of 70 mm, can be seen in the figure. The apple was then immersed in nitrogen gas for about 24 hours, by placing it in a plastic bag flushed with nitrogen gas before being sealed, as discussed above. Then it was brought out into ambient air and the re-invasion of oxygen containing ambient air was measured. The same apple was then pealed, immersed in nitrogen gas for 12 hours before the measurement procedure was repeated. Finally, a new apple, with a thickness of 74 mm, was exposed to an atmosphere with a higher concentration of oxygen than in ambient air for about 12 hours, before it was brought out into ambient air and the measurement procedure was again repeated. The time evolution of the oxygen equivalent mean path length was found to approximately follow a simple exponential curve, which also could be expected with the experimental procedures utilised (Sjöholm et al., 2005). The time constant for the re-invasion of oxygen into the nitrogen-exposed apple with skin was found to be around 100 min. It takes approximately 5 hours for the apple to reach the same steady-state oxygen concentration as before the treatment. It can also be seen that in the pealed apple, the concentration equilibrium of oxygen becomes twice as high as before the apple was pealed, and that the gas exchange is three times faster than in an intact apple.

Similar recordings obtained with our new more practical backscattering detection geometry (Fig. 2a) are shown for a 67-mm thick Granny Smith apple in Fig. 4. The same apple was also measured in transmission geometry, rendering a slightly slower change in the gas signal with time, which might be explained by the fact that the probed depth of the fruit is different in the two geometries (Sjöholm et al., 2005).

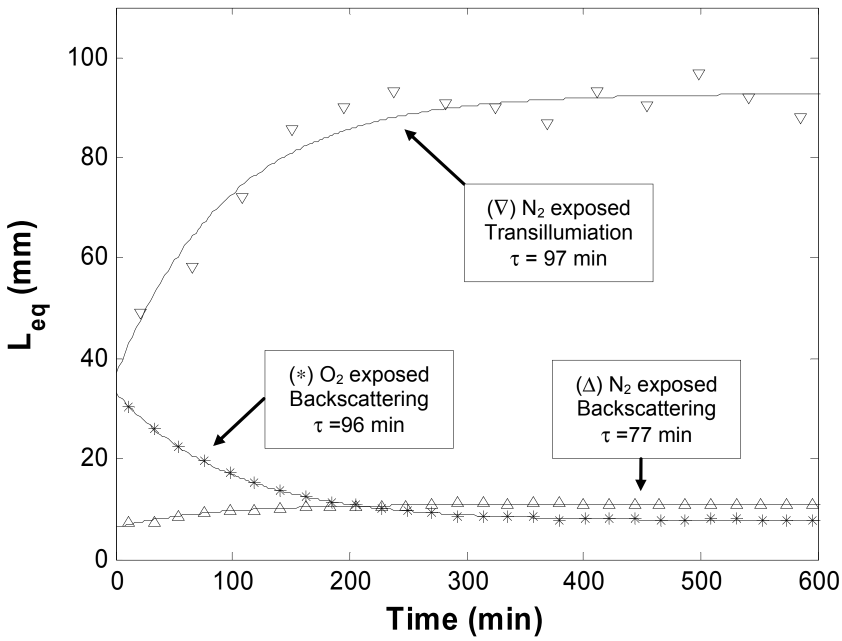

Fig. 4. Measurements of the gas exchange in transillumination and backscattering geometries in ambient air on a Granny Smith apple after different treatments; pre-treated by immersion in nitrogen gas for 24 hours in transillumination (□), pre-treated by immersion in nitrogen gas for 24 hours in backscattering geometry (□), and pre-exposed to an atmosphere with higher concentration of oxygen than in ambient air for 12 hours (). Exponential functions fitted to the data together with the estimated time constants are also included.

The same apple was also studied in backscattering geometry after oxygen exposure. As can be seen in the figure, the gas content equilibrium of oxygen becomes slightly lower, which might be due to the extreme levels of oxygen the apple has been exposed to, which might have harmed the apple. However, the reason can also be due to slightly different probed volumes of the apple.

To illustrate the possibility to use the GASMAS technique for modified atmosphere packaging issues, two different horticultural produces, apple and mushroom, with different expected respiration rates, were sealed in plastic bags and the consumption of oxygen was measured inside the produces in backscattering geometry. The results given in Fig. 5 show, as expected, that the change in the oxygen signal is faster for the mushroom than for the apple, which is a result of the faster respiration rate of the former compared to the latter (Gross et al., 2004).

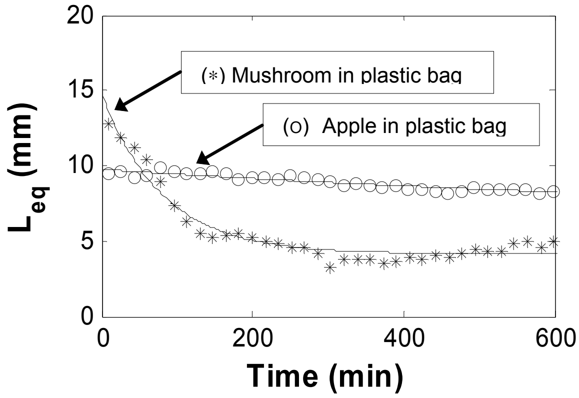

Fig. 5. The time evolution of the oxygen signal for two different horticultural produces with different respiration rates, apple (O) and mushroom (), while sealed in plastic bags, measured in backscattering geometry.

Finally, we illustrate some preliminary measurements in backscattering geometry on other types of horticultural produces such as avocado and orange. The GMS values from three avocados with different maturity are shown in Fig. 6. The hardness of the avocados was measured with a Stevens LFRA Texture Analyzer equipped with a circular probe with a diameter of 25 mm. Each avocado was compressed about 2 mm with a probe speed of 0.2 mm/s while the maximum force was measured. With increasing maturity it is noticed that the GMS values are reduced, probably related to both lower gas contents and less scattering. These aspects could be elucidated in time-resolved measurements (Somesfalean et al., 2002).

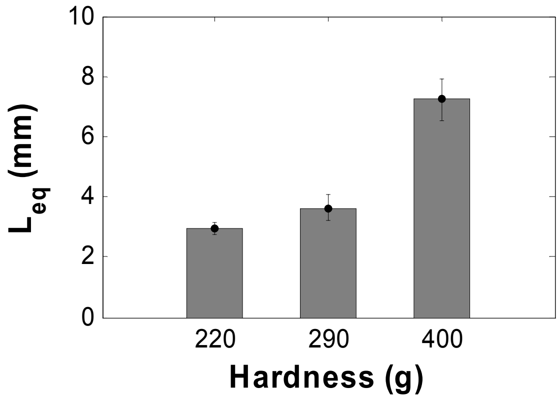

Fig. 6. The equivalent mean path length, Leq, measured for three avocados with different maturity, in backscattering geometry. Four measurements were performed on each avocado and the averages together with error bars corresponding to one standard deviation are shown.

Gas exchange into an orange, with and without peal, was measured after nitrogen exposure. In Fig. 7 the diffusion curve for the intact orange is shown, where the time constant was estimated to approximately 2.5 hours. No change in the GMS values with time could be observed for the equally treated pealed orange, which could be due to the low signal obtained compared to the background noise. However, the results indicate the possibility to study oxygen dynamics in the peal of an orange.

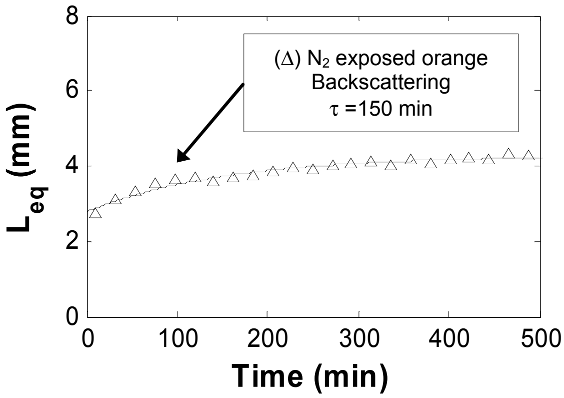

Fig. 7. Measurements of the gas exchange in backscattering geometry on an orange; intact orange pre-treated by immersion in nitrogen gas for 24 hours. Exponential function fitted to the data together with the estimated time constant is also included.

CONCLUSION

As illustrated by the preliminary work reported in the present paper and by Persson et al. (2005) the GASMAS technique has potential to non-intrusively and in real-time provide information on the conditions of horticultural produces. In contrast to other techniques, the gas is measured non-intrusively inside the tissue rather than as gas extracted from the fruits. Applications regarding basic plant physiology as well as in controlled atmosphere fruit storage and in modified atmosphere packaging processes can be foreseen. Also different stages of processing, including fermentation, could be studied. We presently aim at developing compact and realistic equipment for GASMAS studies also in the field.

ACKNOWLEDGEMENT

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Etude des échanges gazeux dans les fruits par des techniques de spectroscopie laser

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Résumé

Des mesures temps réel et non intrusives du taux et de la diffusion d'oxygène dans des fruits sont présentées. Elles sont basées sur des techniques de spectroscopie laser, qui observent les caractéristiques d'absorption dues aux gaz que les fruits échangent par les pores. Cette technique, appelée GASMAS, utilise des diodes laser ajustables. Comme application, les transports d'oxygène dans les pommes et dans les oranges sont mis en évidence et évalués. Cette technique peut également être utilisée pour caractériser des atmosphères modifiées, comme celles de l'alimentaire, comme le montre l'application sur des produits horticoles. D'autre part, des études préliminaires pour estimer la maturité des avocats, de manière non intrusive, sont présentées. Cette technique dispose d'un potentiel intéressant pour le développement de systèmes compacts susceptibles de fournir des nouveaux types d'information pour la gestion post récolte des fruits.