Appl. Phys. B 79, 793–797 (2004)
DOI: 10.1007/s00340-004-1627-y

Applied Physics B
Lasers and Optics

X. GUO[1]
D. BICANIC[2,3,✉]
R. IMHOF[1]
P. XIAO[1]
J. HARBINSON[4]

Optothermal transient emission radiometry for studying the changes in epidermal hydration induced during ripening of tomato fruit mutants

[1] South Bank University, School of EEIE, 103 Borough Road, London SE1 0AA, UK
[2] Laser Laboratory for Photothermal Science, Department of Agrotechnology and Food Sciences, Biophysics Division, Wageningen University and Research Centre, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands
[3] Department of Human Nutrition and Food Chemistry, Faculty of Biotechnology and Food Technology, Zagreb University, Pierotijeva 6, Zagreb, Croatia
[4] Horticultural Production Chain Division, Department of Plant Sciences, Wageningen University and Research Centre, Marijkeweg 22, 6704 PG Wageningen, The Netherlands

Received: 26 March 2004/Revised version: 28 July 2004
Published online: 25 August 2004 • © Springer-Verlag 2004

ABSTRACT Optothermal transient emission radiometry (OTTER) was used to determine the mean surface hydration and the hydration profile of three mutants (beefsteak, slicing and salad) of harvested tomatoes (*Lycopersicon esculentum*) that were kept under ambient conditions for as long as 51 days. Maximal sensitivity of OTTER to water in the samples was achieved by using 2.94 μm and 13.1 μm as excitation and emission wavelengths, respectively. The surface hydration increases rapidly and reaches a constant level during the remaining period. The hydrolysis of pectic substances that occur in tomatoes while ripening might be a possible cause for the observed change in hydration.

PACS 81.70.Cr; 39.30.+w; 87.14.-g; 78.47.+p

1 Introduction

The shelf life of harvested tomatoes depends on the extent of maturity at the time of their picking and the storage of tomatoes rarely exceeds a period of several weeks [1, 2]. The progressively increasing softness of the tissue is one of the recognizable changes that accompanies the process of ripening. It is anticipated that the transformation of rigid cell walls into a flimsy arrangement of weakened cell walls and hydrated cellular matter is caused by the hydrolysis of pectins and other constituents of the cell walls [3].

Continued demand for the quality of agricultural commodities is the reason for perpetual development of new, reliable, non-destructive (ND) and objective methodologies for assessing some aspects of food quality. An example of the ND method used to analyse intact fruit is VIS-NIR spectrophotometry that relates the content of water and sugar to specific spectral regions [4].

Optothermal transient emission radiometry (abbreviated OTTER) is a non-contact and non-destructive inspection method [5]. Recently OTTER, applied for the first time for analysis of food specimens, was shown capable of assessing the depth-dependent hydration profiles in the skin of grapes [6]. The research study, the results of which are described in the paper presented here, focuses on the investigation of changes in the epidermal hydration occurring during the ripening in three varieties of harvested tomato fruit mutants stored under ambient conditions for as long as 51 days.

Briefly, what OTTER detects is the variation in the amount of infrared (IR) thermal radiation emitted by the selectively absorbing test sample after exposing the latter to pulsed laser radiation of the appropriate wavelength. In general, the detected OTTER signal contains information about both the optical and thermal properties of the test sample. A typical time dependence of the OTTER signal obtained from a homogeneous, semi-infinite sample is shown in Fig. 1. The two independent decay times are obtained when the least-squares method is used to fit such an impulse response curve. Both of these are absolute parameters of the signal and hence insensitive to minor displacement of the sample and to other instrumental effects [5].

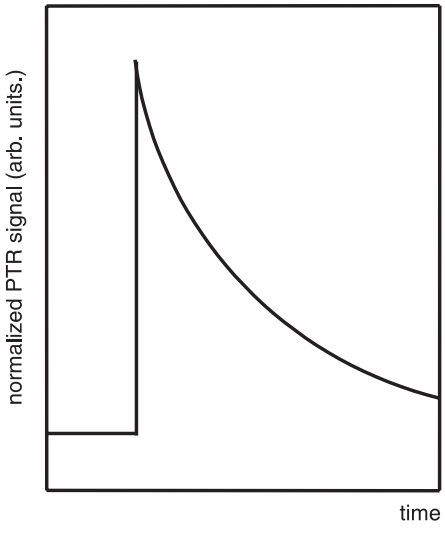

FIGURE 1 Typical shape of the normalized signal obtained in OTTER measurements from a semi-infinite and homogeneous sample (the label abr. refers to arbitrary units)

✉ Fax: +31-317-482725, E-mail: dane.bicanic@wur.nl

To properly interpret hydration data collected in OTTER measurements it is necessary to have (i) a mathematical model valid for a semi-infinite homogeneous sample, and to assume (ii) that the following two approximations are simultaneously being satisfied: (a) the sample's absorption coefficient α at the excitation wavelength is much larger than the absorption coefficient β of the sample at the emission wavelength and (b) the absorption coefficient β is very sensitive to the change of hydration H [7–9]. It is under these conditions that two decay times that are normally abstracted from the OTTER signal reduce to a single time τ. Theoretical analysis shows that the hydration H of the sample is approximately equal to the reciprocal of τ [10]. As real samples are rarely truly homogeneous, H measured via OTTER actually represents the mean hydration of the sample (i.e. the level of hydration within a depth that is actually being probed) and can be calculated from the relationship

$$H \propto \frac{1}{\sqrt{\tau}}, \tag{1}$$

with τ acquired by least-squares fitting [10] the transient curve.

The so-called segmented least-squares (SLS) approach was developed [11] to deal with the OTTER signals obtained from inhomogeneous samples and to calculate depth-dependent hydration profiles. The SLS approach relies on the assumption that, following excitation, OTTER signals originating from various depths in the sample will emerge at varying time intervals. The signal versus time plot is then divided into small segments each of which is least-squares fitted itself. If the sample is thermally homogeneous, one can obtain the depth-dependent information about β and hence also about the depth profile of hydration [12].

2 Experimental

2.1 *Apparatus*

Figure 2 shows the schematic diagram of the experimental set-up used for OTTER measurements on raw tomatoes. A Q-switched Er:YAG laser emitting 2.94-μm radiation that coincides with the strongest absorption peak of water was used for the excitation. An interference filter placed in front of a liquid nitrogen cooled HgCdTe detector sets the detection wavelength to 13 μm. Data collected during 40 s (after 200 consecutive laser pulses) was averaged to improve the signal to noise ratio.

2.2 *Samples*

Three tomatoes (*Lycopersicon esculentum*), i.e. beefsteak tomato (large and hard, grown in Spain), salad tomato (small and soft, grown in the UK) and slicing tomato (large and hard, from Holland) were studied during 51 and 25 days. The tomato fruits were kept in the laboratory environment (room temperature) during the entire period of investigation. Measurements were performed each day; to reduce possible effects of inhomogeneity, care was exercised to irradiate consistently the same region on the tomato. As it was impractical to interrogate several locations on the sample in order to obtain error bars due to the sample's curvature,

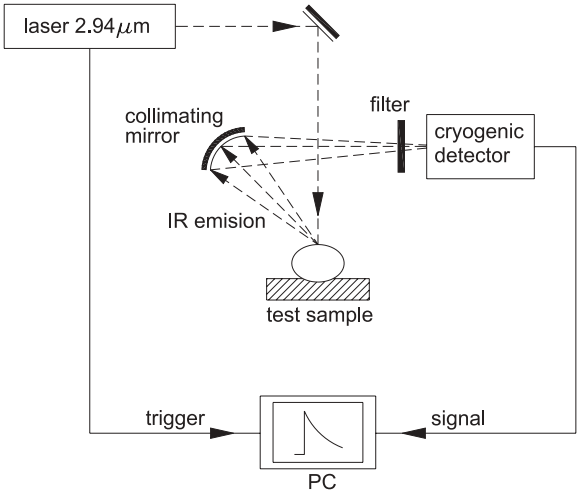

FIGURE 2 The experimental set-up used in OTTER measurements. The plane mirror diverts the pulsed laser radiation onto a test sample positioned on the adjustable platform. The IR radiation emitted by the sample as a result of transient heating is collected using the ellipsoidal mirror and then focused onto the cryogenic infrared detector provided with an interference filter. The detected signal is amplified and sent to a transient digitizer. Finally, digitised data are displayed on the oscilloscope and analysed by a personal computer (PC)

a hundred successive measurements on a selected spot have been performed consistently; the signal to noise ratio reached 200.

3 Results and discussion

Mean surface hydration and the hydration depth profiles acquired from the three tomato mutants are shown in Fig. 3. The variation of the mean surface hydration in a beefsteak tomato during 51 days after the purchase is displayed in Fig. 3 (top). A sharp increase (about 70% of maximal level) in the hydration is observed during the initial five days; for longer periods the increase of hydration is less pronounced. Figure 3 (bottom) shows the hydration depth profiles for a beefsteak tomato recorded at days 1, 11 and 51; the hydration increases with depth. The change of hydration with depth (i.e. the slope) appears to be similar for the three lines (Fig. 3, bottom). However, the surface hydration itself varies, being the lowest on the first day and reaching the highest value at the very last day. Furthermore, the gradient of the surface hydration during the initial 10 days is larger than that found for the remaining period of 41 days. This result is in agreement with data obtained for the mean surface hydration (Fig. 3, top), implying an overall increase of hydration when moving from the surface to some depth beyond the surface. At the signal to noise ratio of 200, the calculated error for data shown in Fig. 3 (top and bottom) is 0.5%.

Figure 4 (top) shows changes of mean surface hydration of a salad tomato during the experiment. Similar to what was observed in the case of a beefsteak tomato, the hydration increases sharply (reaching about 80% of its final value) within only four days; not much change is observed after the sixth day. Figure 4 (bottom) shows the hydration depth profiles for a salad tomato on days 1, 11 and 51 from the onset of the experiment. The hydration increases with depth at a compara-

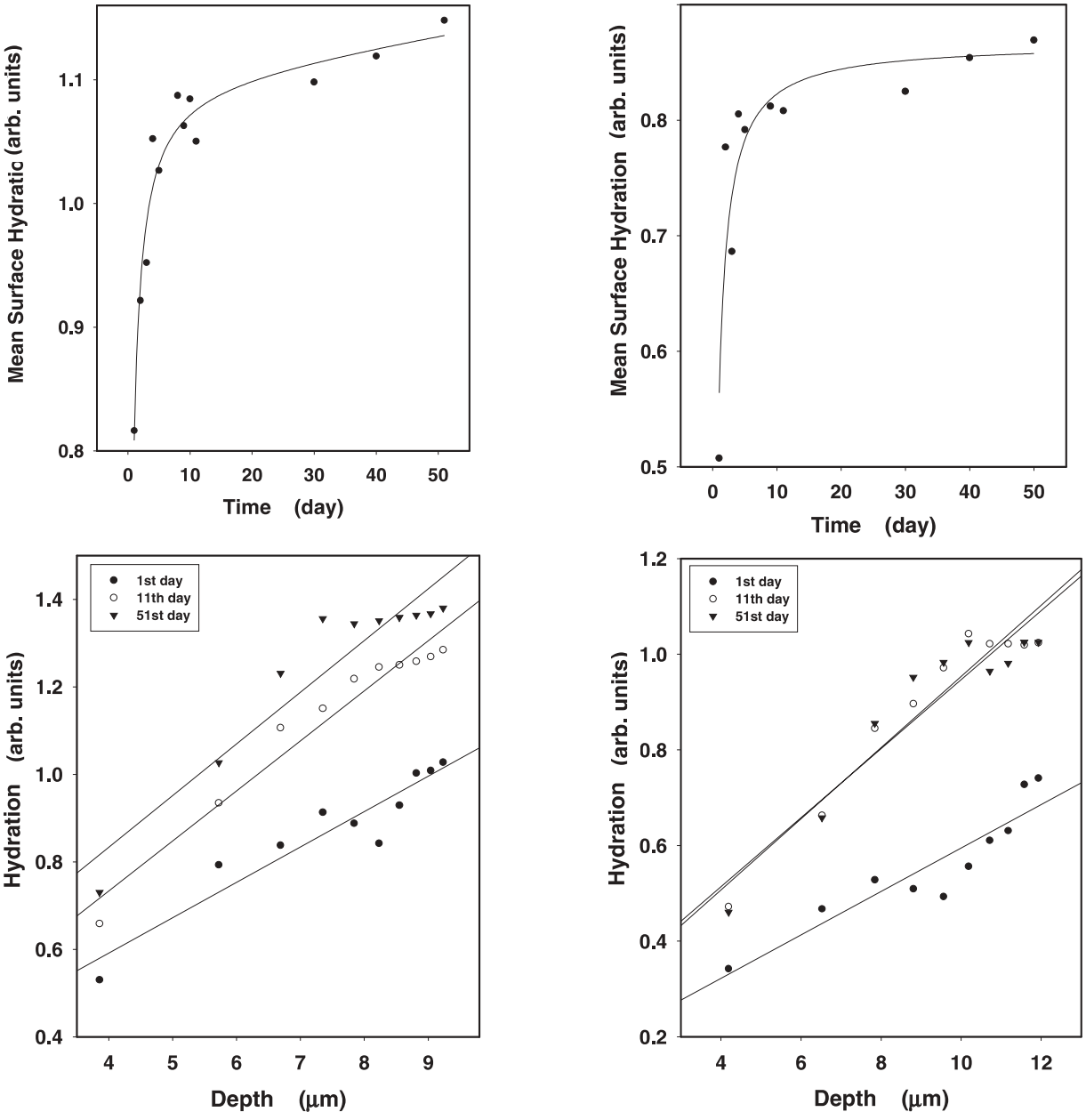

FIGURE 3 Time-dependent surface hydration (*top*) and hydration depth profile (*bottom*) of beefsteak tomato

FIGURE 4 Time-dependent surface hydration (*top*) and hydration depth profile (*bottom*) of salad tomato

ble rate. The nearly overlapping depth profiles (11th and 51st days) and the mean surface hydration data suggest no change in the overall surface hydration of the tomato during the final 40 days.

Figure 5 (top) displays the time dependence (25-day period after purchase) of a mean surface hydration for a slicing tomato. With the exception of the first two days, only a slight change in hydration was observed. The hydration depth profiles (Fig. 5, bottom) at days 1, 11 and 22 overlap and confirm the mean surface hydration results obtained by OTTER measurements. Again, just as in Figs. 3 and 4, the calculated error for data shown in Fig. 5 (top and bottom) is 0.5% at the signal to noise ratio of 200.

The greatest depth of measurement achieved by this OTTER experiment is about 12 μm; this implies that phenomena observed occur in the outermost layer of the tomato fruit. This latter is a poorly structured epidermal layer that comprises a cuticle and remnants of cell walls derived from epidermal cells destroyed during the expansion of the fruit. The depth of this epidermal layer is variable and extends from 4 μm up to about 20 μm; consequently, the hydration state changes recorded by the OTTER technique are occurring within this layer. The epidermal layer is underlain by a hypodermis of small flattened living cells (which individually have a depth of about 10 μm), which may also become cuticularised. Changes in hydration state recorded with OTTER occur within the epidermal layer and possibly (depending on the thickness of the epidermis) also in the hypodermis (underlain by progressively larger isodiametric parenchymatous cells).

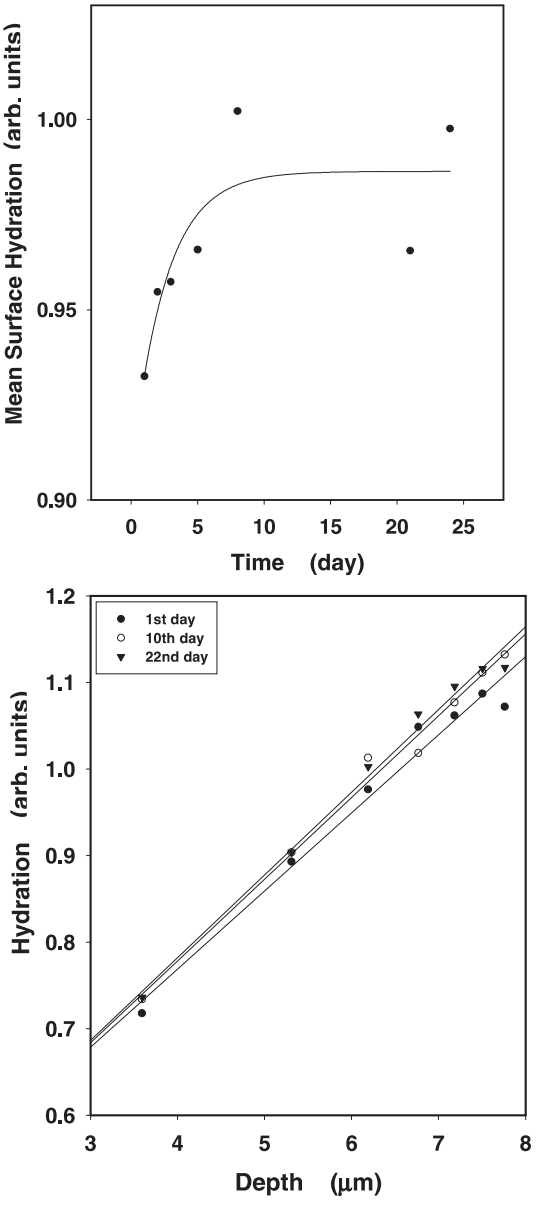

FIGURE 5 Time-dependent surface hydration (*top*) and hydration depth profile (*bottom*) of slicing tomato

The cuticle component of the epidermal layer is composed largely of waxes; in plants these are esters of long-chain fatty acids and alcohols. These are hydrophobic components that make epidermal tissue relatively impermeable to water loss. In combination with the underlying cells, the cuticle also forms a tough, slippery skin that mechanically protects the plant tissue from attack by fungi, insects and other pests. The cell-wall fragments found alongside the cuticle are mainly composed of cellulose and other long-chain carbohydrates; these are hydrophilic in nature.

There is a clear gradient in the hydration of the outer 8 μm (Fig. 5), 10 μm (Fig. 3) or 12 μm (Fig. 4) of the fruit; the average degree of hydration of this epidermal layer increases with the time of ripening. The increase in hydration with depth is due to a high relative water content of the hypodermal layer of the underlying tissues and the equilibration of this water with the carbohydrate of the cell-wall remnants embedded in the outer epidermal layer. It is unclear whether the decrease in hydration towards the surface of the fruit is simply due to evaporative water loss from the outer surface of a homogeneous epidermal layer (thus producing a decrease in hydration across the layer) or an increasing content of hydrophobic material towards the outer surface of the epidermis.

Considerable differences in the time-dependent changes of the extent of hydration are observed between the beefsteak (Fig. 3) and salad (Fig. 4) tomatoes on one hand and the slicing tomato (Fig. 5) on the other. The beefsteak and salad tomatoes show marked increases in both the average surface hydration and the hydration depth profiles for longer times. On the other hand, the slicing tomato exhibits only a small increase in the average surface hydration while the hydration depth profile remains largely unchanged with time. Two main processes are likely to cause the change in the surface hydration of beefsteak and salad tomatoes. First, enzymic softening and the breakdown of cell walls in the bulk tissue of the ripening tomato fruit result in the release of aqueous cell contents and, second, the enzymic modification of the carbohydrate components in the outer epidermal layer leads to the softening of this material and still further hydration [13, 14].

Softening of fruit tissue during the ripening process is due to the modification of the pectins and hemicelluloses, both of which are cell-wall carbohydrates. A major (but not the exclusive) role during cell-wall softening is attributable to the enzyme polygalacturonase: tomato fruits from plants generically modified to produce greatly reduced amounts of this enzyme (specifically the endopolygalacturonase) soften more slowly than wild-type fruits and thus have a longer shelf life. The softening process is accompanied by the thickening and swelling of the cell wall due to an increase in its hydrophilic character as the pectins are hydrolysed to shorter-chain molecules. It is probably this process that occurs in salad and beefsteak tomatoes and produces an increase in hydration. Given the cuticularised, hydrophobic nature of the outer epidermal layer, softening and swelling of cell-wall material embedded in this cuticle will increase the average hydration of this region of the fruit, thereby generating the observed phenomenon. Such a hydration process would be facilitated by wall softening and cell rupture in the parenchymatous mass of tissue that makes up most of the tomato pericarp. This again would allow the release of the aqueous cell contents from these cells. The smaller changes in hydration found in the slicing tomato may reflect the firmer nature of this type of fruit, necessary for mechanical resistance to the stresses imposed by the slicing process.

4 Conclusions

The OTTER technique was used to monitor hydration and determine hydration depth profiles in three tomato mutants kept in the laboratory for defined time periods. For all tomatoes investigated in this study it was found that the surface hydration increases during the first few days and then stabilizes for the remaining period; this is likely related to the ongoing ripening process in tomatoes. The outcome of the above studies suggests the potential of OTTER for the ND, non-contact, real-time monitoring of maturation level in tomatoes and other harvested fruits.

REFERENCES

1 D. Grierson, G. Tucker, N. Robertson: 'The Regulation of Gene Expression during the Ripening of Tomato Fruits'. In: *Quality in Stored and Processed Vegetables and Fruit*, ed. by P.W. Goodenough, R.K. Atkin (Academic, London 1981) pp. 179–181

2 E.W. Simon: 'Membrane Changes Underlying Post-harvest Deterioration in Quality'. In: *Quality in Stored and Processed Vegetables and Fruit*, ed. by P.W. Goodenough, R.K. Atkin (Academic, London 1981) pp. 247–252

3 R.J. Redgwell, E. MacRae, I. Hallett, M. Fischer, J. Perry, R. Harker: Planta **203**, 162 (1997)

4 A.D. Mowat, P.R. Poole: J. NIR Spectrosc. **5**, 113 (1997)

5 R.E. Imhof, D.J.S. Birch, F.R. Thornley, J.R. Cilchrist, R. Strivens: J. Phys. E: Sci. Instrum. **17**, 521 (1984)

6 X. Guo, D. Bicanic, K. Keijser, R.E. Imhof: Eur. Food Res. Technol. **217**, 263 (2003)

7 R.E. Imhof, B. Zhang, D.J.S. Birch: 'Photothermal Radiometry for NDE'. In: *Non-Destructive Evaluation*, ed. by A. Mandelis (Prentice-Hall, Englewood Cliffs, NJ 1994) pp. 185–236

8 R.M.S. Bindra, R.E. Imhof, P. Xiao, J.J. Andrew: SPIE Proc. **2395**, 566 (1995)

9 P. Xiao, R.E. Imhof: SPIE Proc. **2681**, 31 (1996)

10 P. Xiao, R.E. Imhof: SPIE Proc. **2970**, 276 (1997)

11 P. Xiao, R.E. Imhof: UK Patent Application No. 0004374.5 (2000)

12 P. Xiao, R.E. Imhof: *Skin Bioengineering Techniques and Applications in Dermatology and Cosmetology* (Curr. Probl. Dermatol. **26**) (Karger, Basel 1998) p. 48

13 M. Knee, I.M. Bartley: 'Composition and Metabolism of Cell Wall Polysaccharides in Ripening Fruits'. In: *Recent Advances in the Biochemistry of Fruit and Vegetables*, ed. by J. Friend, M.J.C. Rhodes (1981) p. 133

14 G.E. Hobson: 'Enzymes and Texture Changes during Ripening'. In: *Recent Advances in the Biochemistry of Fruit and Vegetables*, ed. by J. Friend, M.J.C. Rhodes (1981) p. 123