

Technical paper

Practical, Reliable and Inexpensive Assay of Lycopene in Tomato Products Based on the Combined Use of Light Emitting Diode (LED) and the Optothermal Window

Dane Bicanic,^{1,2} Ruud Cuypers,¹ Svjetlana Luterotti,³
Maja Šporec,¹ Angela Zoppi⁴ and Jasminka Vugec³

¹Laboratory of Biophysics, Agrotechnology and Food Sciences, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

²Faculty of Food Technology and Biotechnology, University of Zagreb, Pierotijeva 6, Zagreb, Croatia

³Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, Zagreb, Croatia

⁴European Laboratory for Non-linear Spectroscopy, LENS, University of Florence, via Nello Carrara 1, Sesto Fiorentino, Italy

* Corresponding author: E-mail: dane.bicanic @wur.nl

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Abstract

Light emitting diode (LED) combined with the concept of optothermal window (OW) is proposed as a new approach (LED-OW) to detect lycopene in a wide range of tomato-based products (tomato juice, tomato ketchup, tomato passata and tomato puree). Phytonutrient lycopene is a dominant antioxidant in these products while beta-carotene is present in significantly lower quantities. Therefore for all practical reasons the interfering effect of beta-carotene at 502 nm analytical wavelength can be neglected. The LED-OW method is low-cost and simple, yet accurate and precise. The major attributes of the new method are its rapid speed of response and the fact that no preparation whatsoever of the sample is needed before the analysis. The lycopene found in tomato products studied here varies from 8 mg/100 g to 60 mg/100 g fresh product. Results obtained by LED-OW method were compared to the outcome of conventional, time consuming spectrophotometric methods and the correlation was very good ($R \geq 0.98$). Precision of the LED-OW instrumental set-up ranged from 0.5 to 7.4%; the RSD achieved for lycopene-richest samples (≥ 40 mg/100 g) did not exceed 1.7%. Repeatability of analysis by LED-OW was found to vary between 0.7 and 7.1%.

Keywords: Lycopene, LED, optothermal window, tomato products

1. Introduction

The effect of specific food ingredients on human health is becoming steadily more recognized. As an example, outcomes of various epidemiological studies provide the evidence that individuals consuming foods rich in carotenoids have lowered risk of developing certain types of diseases including the reduced incidence of some cancers. Lycopene occurs naturally only in plant and algal tissues. In particular, the reports on a wide range of health benefits of anti-oxidant lycopene (high single-oxygen quenching rate) from tomatoes (most abundant sources of lycopene)

and foods derived from tomatoes have kept these products in the public eye. Lycopene content of a tomato depends on the cultivar and the degree of fruit ripeness at the time of harvesting. Other sources of this phytonutrient include watermelon, guava, red grapefruit and papaya.

Difficulty often encountered in studies of tomatoes and tomato-based products is the quantification of lycopene.^{1,2} High pressure liquid chromatography (HPLC) and spectrophotometry (SP) are currently the two most frequently used methods for determination of lycopene. The HPLC offers superior performance in terms of accuracy, sensitivity and specificity in detecting lycopene in a com-

plex matrix. However, the same method is not only time consuming but it also requires a skilled labor. In addition, the eluent and chemicals used to extract the carotenoids pose a certain hazard to the experimentalist; similar arguments apply to SP as well. Obviously, there is a need for a low-cost, fast, sensitive, specific and non-destructive methods capable of detecting lycopene with a minimum of sample preparation or no preparation at all.^{3,4}

Recently, it was demonstrated that concept of optical window (OW) combined with the c.w. argon-ion laser emitting at 502 nm can be regarded as a novel approach towards detection of lycopene in the tomato-based products.⁵ The main reasons for selecting 502 nm as the analytical wavelength are the selectivity and sensitivity of lycopene detection achieved at this wavelength, namely a relatively high spectral contrast between lycopene (the dominating carotene) and β -carotene (the minor carotene). The OW approach allowed for a direct determination of lycopene in a wide range of opaque samples ranging from a tomato juice (about 8 mg lycopene per 100 g wet mass) to a triple concentrated puree (about 70 mg lycopene per 100 g wet mass).^{5,6} Very good agreement was observed between OW data (when Ar ion laser was used as an excitation source at 502 nm) and results obtained from the very same samples by the well-established analytical methods such as spectrophotometry⁵ and HPLC.⁶

Although, the OW concept quoted above represents undoubtedly an important step forward in facilitating detection of lycopene in tomato-based foods, the relatively high investment cost of the laser and the size of the instrumentation preclude, for the time being, a widely spread use of this technique in a daily practice. Therefore, a new research study was undertaken with the main objective to develop a OW based lycopene detector that, instead of the argon laser, makes use of a light emitting diode (LED) as a intense, compact, low-cost radiation source. In this research study the overall performance of the newly proposed LED-OW device was evaluated and compared to that achieved from the same test specimens by absorption spectrophotometry.

2. Experimental

Fifteen tomato-based products (they were assigned letter codes from A to O) were purchased in different grocery stores; the expiry date reported on the product label was carefully observed. The samples were kept in the refrigerator until actual use. The products included one tomato juice (A), three ketchups (B, C, D), four single concentrated tomato purees (E, G, H, I), one tomato passata (F), three double tomato puree concentrates (K, L, M) and three triple concentrated purees (J, N, O), respectively.

The SP analyses of lycopene in above mentioned products were performed by two different methods, reported previously.^{5,7}

Figure 1 shows the exploded view of LED-OW experimental set-up used in this study. The radiation source [A] is Luxeon star-/c LXHL-LE5C Cyan lumiLED with $\Delta\lambda_{1/2}$ bandwidth of 30 nm. This LED emits between 470 and 530 nm and its spectral power output peaks at 505 nm. This is very close to 502 nm wavelength at which the spectral contrast for quantification of lycopene is optimal. Function generator (0.1 Hz-2 MHz) Philips PM5131 was used to electronically modulate the LED radiation.

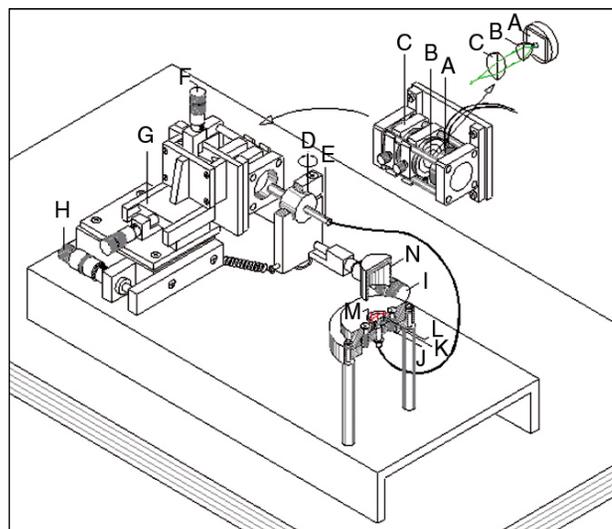


Fig. 1: The experimental set-up used for LED-OW measurements. For in-extenso description of various parts (A to N) of the set-up reader is referred to the text.

Total power emitted by this LED is 70 mW as measured by Spectral Physics 401 power meter when the latter was placed directly in front of LED itself. In an attempt to obtain the quasi-collimated beam (thereby reducing LED's intrinsic divergence) from a strongly divergent LED, the LXHL-NX05 lens [B] from Luxeon, was mounted to the LED; the symmetry axes of LED and lens coincided in space. The plan-convex lens [C] from Melles-Griot (focal length 19 mm, diameter 25.4 mm) was added to focus the quasi collimated LED beam into optical fiber [E] 3 mm in diameter (see also insert in Fig. 1). The position of the lens [C] relative to the fiber's front surface could be adjusted by means of three linear translation stages [F, G and H] stacked atop each other and equipped with the Mitutoyo precision micrometer (linear travel 20 mm and 5 microns resolution). In addition, turning on the micrometer [I] causes the plate [D] accommodating the fiber to rotate about vertical symmetry axis. This in turn enables one to vary the tilt angle subtended by the incident beam and the normal to fiber's [E] front surface facilitating the optimization of coupling efficiency. All optical and mechanical components were mounted on the aluminum block (dimensions: length 300 mm, width 200 mm

and height 80 mm); the entire unit is compact, reasonably light and also portable.

The OW sensor itself is a disk of sapphire (300 microns thick, 16 mm diameter) with the annular ring [K] from a piezoelectric material glued to its bottom. The optical fiber [E] conveys the LED radiation to the OW sensor. The other end of fiber [E] was pressed gently against the bottom of a flat cut Plexyglass cone [J] that fills a space between sapphire disk and the ring [K]. The upper surface of cone is flat (main function of the cone is to reduce scattering) and brought into contact with a rear side of a sapphire disk. The LED radiation enters the sapphire disk from below, passes through it and eventually reaches the test sample deposited atop the disk. Not more than 1.8 mW from the originally emitted 70 mW LED power reaches the sample; presumably such a large loss is the result of poor coupling of LED radiation into both, the fiber and the disk.

In a typical LED-OW measurement tomato product under investigation was poured in a beaker and manually mixed before placing it atop the surface [L, M] of sapphire disk. As to the analysis itself, 2 mL aliquot of product is thought adequate. This is so because the sample being investigated must cover only a central portion (4 mm in diameter) of sapphire disk through which the sample is being irradiated. The effective sampling depth is equal to a thermal diffusion length μ ; at the modulation frequency of 25 Hz thermal diffusion length in tomato-based samples investigated here, is about 50 to 70 microns. The LED-OW concept offers an attractive advantage in a sense that the quantity of sample is not critical as long as certain requirements (see below) are being fulfilled. This is entirely different from what is encountered in the conventional spectroscopy where actual sampling pathlength is controlled by the spacer of appropriate thickness.

The experimental LED-OW set-up is susceptible to the effect of “external” noise caused by vibrations (transmitted through the floor and table) as well by the laboratory airflow; shielding of the sample is therefore recommended. The 150 mm long cylinder 3 mm in diameter mounted coaxially with sapphire disk proved efficient in reducing disturbing effects of air movement.

Detailed explanation of the mechanism responsible for the generation of the OW signal is given elsewhere.^{8–10} For further processing the output signal from the OW-LED sensor is fed to the signal input of Stanford SR830 DSP two phases lock-in amplifier provided with a built-in pre-amplifier ($10^8 \Omega$ feedback resistance). The reference input for the lock-in amplifier was derived from Philips PM5131 function generator. At a selected modulation frequency (f) of 25 Hz, in tomato-based samples μ is shorter than the optical penetration depth $1/\beta$ (β is absorption coefficient at excitation wavelength) and physical thickness L_s of the sample. When the condition $\mu < 1/\beta < L_s$ is met, the generated signal is linearly proportional to the product $\beta\mu$.

3. Results and Discussion

3. 1. Performance of LED-OW

Powerful and stable radiation sources are preferred for LED-OW studies. To check the suitability of the LED used here, its output power level was monitored for one hour. Under given operating circumstances the observed drift was less than 0.3% indicating a very good long-term output power stability.

The objective of the next experiment was to check the frequency response of the present set-up for LED-OW measurements. The measurements were performed with a black drawing ink used as a strong absorber. The magnitude of LED-OW signal (integration time of the lock-in amplifier was 300 ms) generated when irradiating the ink with 1.8 mW LED power, was investigated as a function of the modulation frequency in 1 to 300 Hz range. Data obtained (Fig. 2) shows that only above 20 Hz the log signal-log frequency plot is a straight line (slope of -1 as predicted by the OW theory for a strongly absorbing specimen). The vertical line in Fig. 2 refers to 25 Hz modulation frequency at which actual LED-OW studies of tomato-based products (see below) have been performed. Flattening of the response for ink at frequencies below 20 Hz in Fig. 2 is perhaps due to the fact that OW's own impedance, just as the LED-OW signal, also exhibits $1/f$ behavior. Clearly, at low frequencies the impedance of LED-OW detector becomes very high and in situation like this, it is the input impedance of the lock-in amplifier that is of primary importance.

Ink was then rejected, sapphire disk cleaned, reloaded with distilled water (at wavelengths emitted by this LED, the absorption of water is weak) and LED-OW measurement repeated to get an estimate of the background signal. It is interesting to observe (Fig. 2) that in the case of water, the LED-OW signal vs. frequency dependence is apparently more complicated (LED-OW signal levels off at 2 Hz) as the line is straight over the entire range of modulation frequencies – no explanation for this can be given (yet). The ratio of the LED-OW signals obtained from black ink and water at a specific frequency, can be regarded as a “dynamic range”; at 25 Hz the latter is approximately 60. The sixtyfold difference in the LED-OW signals between the black ink and water may in the first instance appear rather low. In general, the “background” (blank) signal from water originates perhaps from the LED radiation scattered on the piezoelectric annular ring glued to the rear face of sapphire disk. To minimize the effect of such undesirable phenomenon, the sapphire disk is normally provided with a protective “shield” (conically shaped ring mounted along the entire circumference of piezoelectric material). However, in this study a different approach has been used. As stated before (refer to section Experimental), the transparent cone of Plexyglass (see part J in Fig. 1) was glued to the back surface of the sapphire disk. The outside surface of Plexyglass cone was

painted black to reduce the effect of the scattered light. Although such construction is generally known to produce lowest background signal, it becomes less suitable when optical fiber is used to transport light (as it is being done in this LED-OW experiment). In present LED-OW design the other end of optical fiber is brought into contact with a bottom face of Plexyglass cone instead with the bottom of sapphire disk. It is likely that this not only reduces the actual LED power reaching the sample atop the OW disk but also causes a higher background signal. Therefore, future LED-OW experiment in which the presently used fiber will be replaced by the set of mirrors and lenses to couple LED radiation into LED-OW detector provided with Plexyglass cone is certainly worth the effort, as this may enhance the dynamic range.

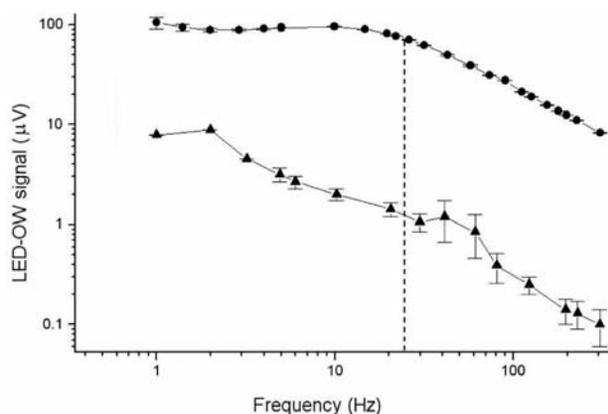


Fig. 2: The log-log plots of the LED-OW signals obtained from the black ink (upper trace) and water (lower trace) versus the modulation frequency. The LED-OW signals were detected at 1.8 mW LED power. Each point represents a mean \pm SD of five consecutive measurements.

The aim of the next measurement was to investigate the proportionality between the magnitude of the LED-OW signal and incident LED power. In doing so, sapphire disk was loaded with the black ink and LED-OW signal measured at 25 Hz while varying LED power between 0 and 1.8 mW. The LED-OW signal was linear ($R^2 = 0.9989$) with the LED power confirming the prediction that the magnitude of LED-OW signal is directly proportional to the amount of heat deposited into sample and hence to the incident power as well.

3. 2. Quantitation of Lycopene in Tomato-based Products by LED-OW

The evidence for very good agreement between the theoretical predictions and the outcomes of several experiments discussed above, suggests that LED-OW can be used to quantify absorption in the opaque samples and

possibly allow (over restricted range of frequencies) for the determination of lycopene in the tomato-based products.

In the final stage of LED-OW experiments the emphasis was therefore on determination of lycopene in fifteen different tomato-based products. All LED-OW experiments were performed at 25 Hz and at room temperature. With the specific product atop sapphire disk, 150 successive amplitude readings of LED-OW lock-in signal were taken over 30 seconds before calculating the corresponding average; resulting standard deviation is regarded as a measure of instrument precision achievable by a “single load” LED-OW experiment. Using the sampling rate of 150 readings in a 30 seconds interval is equivalent to 5 readings in 1 second or a one, single reading taken each 200 ms.

After the first measurement on selected tomato-based product was completed, the sample was rejected, the surface of disk cleaned with ethanol and rinsed with distilled water. Then, a fresh quantity of the same tomato-based product was deposited on a disk and entire procedure repeated; in total three to four such replicate measurements have been performed with each tomato product. Data collected in this manner was used to compute the average signal S_s and analysis repeatability (precision achieved in “multiple load” measurements). Similar procedure was also followed for the black drawing ink. With the average LED-OW values for S_s (for specific tomato-based product) and S_l (for ink) being known, one proceeds by calculating the normalized LED-OW signal S as $S = S_s/S_l$. Under the condition $\mu < 1/\beta < L_s$, the normalized LED-OW signal S depends solely on the product of sample’s absorption coefficient β and thermal diffusion length μ i.e.:¹¹

$$S = \frac{\beta \cdot \mu}{\sqrt{(1 + \beta \cdot \mu)^2 + 1}} \quad (1)$$

In the lin-log plot, S is a sigmoid function of $\beta\mu$. For high $\beta\mu$ values S tends to 1, while in case of low absorption ($\beta\mu \ll 1$), S converges to $S = \beta\mu/\sqrt{2}$. Taking the first derivative of S with respect to $\beta\mu$ [see eq. (1)] and setting it equal to zero, gives $\beta\mu=1$ as a value at which the sensitivity is maximal (the point of inflexion). It was already stated above that the normalized signal S in a typical LED-OW experiment is computed from the LED-OW lock-in signals obtained from a given test sample and a suitable reference (black ink). The corresponding $\beta\mu$ value is then calculated by inverting eq. (1) which yields:

$$\beta \cdot \mu = \frac{2}{\sqrt{(2/S^2) - 1} - 1} \quad (2)$$

Figure 3 features the 100 $\beta\mu$ product calculated from eq. (2) from LED-OW signals obtained experimentally at 25 Hz for a series of tomato-based products. This product

is plotted *versus* the concentration of lycopene determined in the very same products by two spectrophotometric methods.^{5,7} The correlation between $\beta\mu$ product and lycopene concentration not exceeding 40 mg/100 g ($N = 9$) is characterized by $R = 0.987$ and the residual sum of the squares around the line, $RSS = 3.2 \cdot 10^{-4}$. Even with lycopene richest products (up to 60 mg/100 g, $N = 15$), product $\beta\mu$ is still linearly proportional to lycopene concentration; the correlation coefficient $R = 0.979$ ($RSS = 3.4 \cdot 10^{-3}$) is quite high when bearing in mind a wide range of products studied here. The repeatability of LED-OW method expressed as RSD of three to four independent analyses was found to be 0.7–7.1%.

Instrument precision obtained from single sample load ranged between 0.5 and 7.4% (based on 150 consecutive readings of the lock-in amplifier). Good instrument precision (maximum 1.7% RSD) was achieved for samples containing ≥ 40 mg lycopene/100 g product; for samples with lower lycopene content the instrument precision tends to deteriorate. The fact that instrument precision enhances with the increase of the signal is evident from a power function $y = 12.758x^{-1.12}$ between RSD of instrument readings (y , in %) and LED-OW signal (x , in μV). Good correlation ($R = 0.936$) was obtained even when all measurements were considered (total number of points, $N = 69$).

Unlike instrument precision, accuracy of lycopene quantitation deteriorates in the samples with >40 mg lycopene/100 g product.

The linearity range as well as the scatter of data points around the line (and consequently the accuracy in

estimating lycopene content in the sample), depend not only on the concentration of lycopene, but also on the dry tomato-matter content of the sample. Apparently, in a less viscous product (such as passata) it is easier to achieve a good and reproducible thermal contact between the sample and sapphire surface than in a triple concentrated tomato puree characterized by a higher dry matter content. In addition, the quality of thermal contact may be affected by the incomplete homogenization of test products. This in turn might manifest itself in a somewhat higher error of quantitation in highly concentrated tomato-based products too. Double and triple concentrated tomato purees that are prone to a more pronounced scatter of the points around the regression line could be assayed with a reduced accuracy.

3. Conclusion

In the conclusion, by combining the inexpensive LED source and the concept of OW detection, it was possible to selectively quantify lycopene in a wide variety of tomato-based products without a need for sample preparatory steps. The most important advantages of newly proposed LED-OW device are: i) no need for the extraction step prior to the actual measurement, ii) high speed of response, iii) compact size and iv) a reasonably low cost. However, determination of lycopene by LED-OW is not an absolute method, and therefore constructing the calibration curve (such as the one shown in Fig. 3) is a necessity. This latter implies the necessity for the LED-OW signals obtained under well defined experimental conditions, from tomato-based samples the lycopene concentration of which is accurately known (via SP or HPLC measurements). Once such a calibration curve is available, the concentration of lycopene in an unknown tomato-based sample is simply determined from measured $\beta\mu$ values (for this very sample) obtained from a LED-OW experiment performed under the conditions (LED power, modulation frequency, geometry, OW detector) identical to those used to construct the calibration curve.

Overall, the LED-OW approach proposed here converts the quantification of lycopene from otherwise complex and slow procedure to a simple method that requires no reagents and also drastically reduces the analysis time. Loading/cleaning of LED-OW device is fast and the precision of LED-OW analyses is comparable to that achievable by the traditional techniques. These features make the LED-OW method a useful, low-cost analytical tool with a number of potential applications in practice. One among these is the currently ongoing research the aim of which is to determine lycopene in the raw tomatoes and in some tropical fruits. Furthermore, the on-line kinetics study of the beta-carotene in suspension of algae from bioreactors is being anticipated.

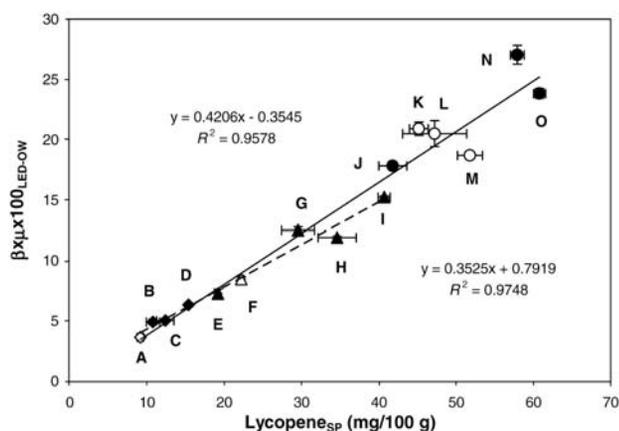


Fig. 3: The hundredfold product $\beta\mu$ obtained from LED-OW measurements plotted *versus* the concentration of lycopene determined by the two spectrophotometric methods (SP) at 502 nm, for various tomato-based products (dotted line: $N = 9$, full line: $N = 15$). The plot was recorded at 25 Hz using 1.8 mW incident LED power. Each point represents a mean \pm SD, $n = 3$ –4 independent analyses by LED-OW, and $n = 2$ –9 independent analyses by SP (\diamond – tomato juice, \blacklozenge – ketchup, \triangle – tomato passata, \blacktriangle – 1x concentrated tomato puree or concentrate, \circ – 2x concentrated tomato puree, \bullet – 3x concentrated tomato puree).

4. Acknowledgement

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Povzetek

Predlagana je nova metoda LED-OW za določevanje likopena v različnih izdelkih iz paradižnika (paradižnikov sok, mezge, paradižnikov pire), ki temelji na tehniki optotermičnega okna (OW) v kombinaciji s svetlečimi diodami (LED). Likopen je prevladujoč antioksidant v izdelkih iz paradižnika, beta karoten pa je prisoten v bistveno nižjih koncentracijah. Zato lahko moteč vpliv beta karotena pri valovni dolžni 502 nm zanemarimo. Metoda LED-OW je enostavna in poceni pa vendar natančna in zanesljiva. Glavna prednost metode, ki ne zahteva posebne priprave vzorca pa je hiter odziv oz. kratek čas analize.

Koncentracije likopena v analiziranih vzorcih so bile med 8 mg/100 g in 60 mg/100 g svežega izdelka. Rezultati metode LED-OW so bili primerljivi z rezultati veliko zamudnejše spektrofotometrične metode ($R \geq 0,98$). Relativni standardni odklon meritev z LED-OW instrumentom je bil v območju 0,5–7,4 % in za vzorce z višjo koncentracijo likopena (≥ 40 mg/100 g) ni presegel 1,7 %.