Quantification of lycopene in the processed tomato-based products by means of the light-emitting diode (LED) and compact photoacoustic (PA) detector

2010 J. Phys.: Conf. Ser. 214 012021

(http://iopscience.iop.org/1742-6596/214/1/012021)

Journal of Physics: Conference Series **214** (2010) 012021 doi:10.1088/1742-6596/214/1/012021

Quantification of lycopene in the processed tomato-based products by means of the light-emitting diode (LED) and compact photoacoustic (PA) detector

D. Bicanic[a,c], H. Skenderović[b], K. Marković[c], O. Dóka[d], L. Pichler[b], G. Pichler[b] and S. Luterotti[e]

[a]Laboratory of Biophysics, Wageningen University, Dreijenlaan 3-Transitorium, 6703 HA Wageningen, The Netherlands
[b]Institute of Physics, Bijenička cesta 46, HR-10000 Zagreb, Croatia
[c]Department of Food Quality and Nutrition. Faculty of Food Technology and Biotechnology, Zagreb University, Pierottijeva ulica 6, HR-10000 Zagreb, Croatia
[d]Institute of Mathematics, Physics and Informatics. Universty of West-Hungary. H-9200 Mosonmagyarovar, Deak F. sq. 1, Hungary
[e]Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, HR-10000 Zagreb, Croatia

Corresponding author's e-mail address: Dane.Bicanic@wur.nl

Abstract: The combined use of a high power light emitting diode (LED) and the compact photoacoustic (PA) detector offers the possibility for a rapid (no extraction needed), accurate (precision 1.5%) and inexpensive quantification of lycopene in different products derived from the thermally processed tomatoes. The concentration of lycopene in selected products ranges from a few mg to several tens mg per 100 g fresh weight. The HPLC was used as the well established reference method.

1. Introduction

There is a steadily growing interest toward exploring the role that the antioxidant lycopene plays in the prevention of a variety of nutritional and health issues in humans. Among these is the lowered risk of contracting the cardiovascular diseases and some cancers [1] .

Tomato, watermelon, guava, papaya, rosehip and the red grapefruit are major nutritional sources of lycopene. The latter compound is a dominant carotenoid in tomato (a primary source of lycopene in the western diet) and gives a vibrant, deep red color to this product. The products derived from the thermally processed tomatoes were found particularly rich in lycopene and processing of tomatoes comprises a large volume of vegetable harvested in many different countries. In addition, it has been established that the bioavailability of lycopene from the processed tomato-based foods is higher than that from raw tomatoes.

The importance of lycopene triggered the interest among the breeders, growers and tomato industry processors for the quantification of this phytonutrient. The HPLC and spectrophotometry, two most widely used approaches for the quantification of lycopene are both slow and costly due to a need for the extraction of pigment prior to the actual analysis. Consequently, there are ongoing efforts the

objective of which is to shorten the analysis time, reduce the investment cost and eliminate the need for a skilled labor force. One example is the study initiated to explore the extent of correlation between the concentration of lycopene (determined by the wet chemical methods) in tomato-based products and the colorimetric indices L*, a* and b* (or parameters derived from these quantities) [2]. Furthermore, FT-Raman, ATR/FT-IR and NIR spectroscopy [3] and the fibre optic visible reflectance spectroscopy [4] were proposed for the quantification of lycopene without the need for sample preparation. Bicanic et al. were first investigators to introduce the photothermal methods for quantification of lycopene in the tomato-based foods. The concept of the so called optothermal window (OW) sensor, when used in a combination with the argon ion laser emitting 20 mW at 502 nm, was demonstrated capable of detecting lycopene over a wide concentration range; the method was also successfully validated against the HPLC [5]. However, the large dimensions and the high cost of the laser precluded the use of such approach for variety of practical field. Applications. In an attempt to reduce substantially the cost and to construct a compact size instrument, Bicanic et al. demonstrated the feasibility of the detection system based on the use of the OW detector and the light emitting diode (LED) [6].

Research study described in this paper is concerned with the development and use of yet another simple and inexpensive approach for a rapid assessment of lycopene in processed tomato-based foods such as tomato ketchup, tomato juices, tomato purees and pastes. The proposed approach is based on the combined use of the low-cost LED and the non-resonant photoacoustic (PA) cell. The concentration of lycopene in these products varies from a few mg to several tens of mg per 100 g fresh weight. The conventional HPLC method served as a golden reference technique.

2. Experimental

Figure 1 shows the scheme (left) and the photograph (right) of the experimental set-up used in this investigation. All components of the experimental set-up were mounted onto the optical table. The source of radiation, Osram DT6-52 Dragon LV W5SG LED, is a Lambertian (120^o) emitter (I_F = 350 mA, luminous flux from 18 to 33 lm, input electrical power 7.2 W) of green radiation with (505 ± 6) nm as a dominant wavelength at I_F. The power supply (Optotronic OT9/220-240/350 mA) provided the stabilized current for LED. The 505 nm is close to 502 nm, the wavelength of which the absorbance of lycopene is maximal whilst at the same time the effect of interfering trans-beta-carotene is minimized. The total radiation output power measured (with power meter Thorlabs S20 MM) just above the LED was about 50 mW. The initially divergent radiation emitted by the LED was first collimated by means of a quartz lens (f=40 mm and 40 mm diameter) L_1 (see Fig. 1). After passing through another quartz lens L_2 (f=170 mm, diameter 40 mm) positioned 220 mm away from L_1, the beam was bent at 90^o (reflection at the plane mirror) and directed into a PA cell. The actual power reaching the PA cell was 4.2 mW which is only a fraction of emitted 50 mW.

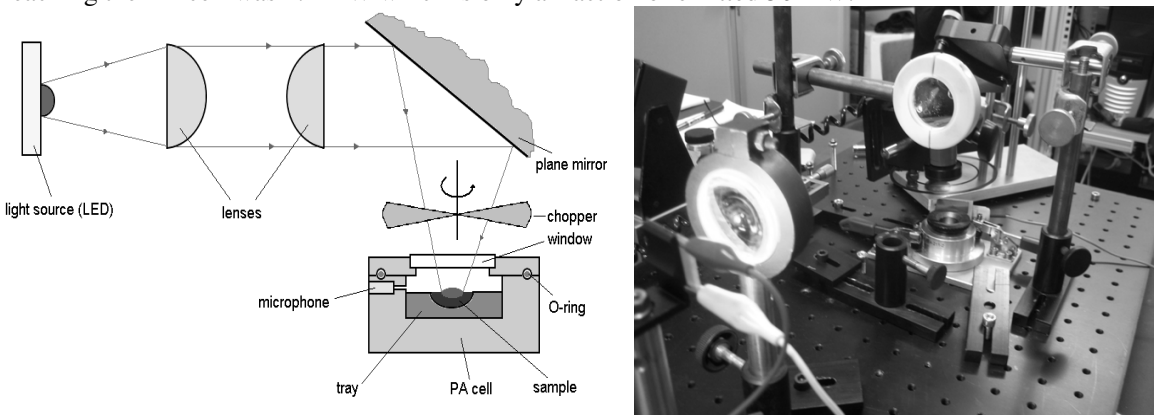

Fig. 1 The schematic diagram of the experimental set-up (left) and the photo of the experimental arrangement (right).

The PA cell itself has the upper and lower section that are easily assembled/dismantled by means of the 10 mm x 2 mm "O" ring (which also provides acoustic seal) and two quick-tightening clamps. The upper section of the PA cell is actually an aluminium housing that accommodates i) a highly transparent, 3 mm thick UV grade synthetic fused silica window 25 mm in diameter (Melles Griot 02WLQ 105) and ii) the condenser microphone Sennheiser KE211-4 characterized by 10 mV/Pa sensitivity across the frequency range extending from 20 Hz to 20 kHz. The lower section of the PA cell which serves as a sample tray, comprises the stainless steel plate (60 mm in diameter and 6 mm thick) provided with a central hemispherical cavity 8 mm in diameter. The tomato product under investigation was transferred into this cavity by means of a small spoon and the excessive quantity removed. Then, the surface of the tomato-based product in a cavity was, by means of a spatula, made flat (flush with the tray).

The air volume of the PA cell (about 150 mm^3) is that of a cylinder defined by the base (cavity 8 mm in diameter) and the distance from the surface of the sample and the rear side of the window (3 mm). In order to get large PA signals, the modulation frequency must be low and the volume of the PA cell must be kept small. Heat losses at the walls are negligible because the height of the PA cell (3 mm) is larger than the thermal diffusion length (about 0.5 mm at 25 Hz) in the air layer above the sample. The signal from the PA cell provided the input for the signal channel of the SRC510 lock-in amplifier. When the incident LED power was 4 mW, the magnitude of PA signal at 25 Hz obtained from a carbon black in sample tray was typically 1.9 mV.

Six processed tomato-based products studied here include two tomato juices, one ketchup, one puree, one double tomato concentrate and one triple tomato concentrate. All products, purchased in supermarkets in Wageningen, The Netherlands and Zagreb, Croatia were kept in the refrigerator and equilibrated to a room temperature before actual measurements. The extraction of carotenoids for HPLC measurements was carried out according to the procedure proposed by Sadler [7]. Four gram homogenized product was transferred into a 100 ml Erlenmeyer flask wrapped in the aluminium foil. The 100 ml mixture of hexane, acetone and ethanol in a volume proportion 50:25:25 was then added and entire flask content agitated again (for 10 minutes, magnetic mixer). Upon adding 15 ml water and following the additional mixing (5 minutes), the organic fraction that contains lycopene was separated from the aqueous phase. The obtained extracts were filtered (pore size 0.45 μm diameter) prior to the HPLC analysis.

Shimadzu LC10AD VP HPLC instrument included a pump Shimadzu LC-10AD-vp, the column oven Shimadzu CTO-10Asvp and Shimadzu SCL-10Avp system controller. The Supelcosil^{TM} LC-18 column (5 μm, 25 cm x 4.6 mm) was maintained at 30°C; the volume injected on the column was 20 μl. The 1:1 mixture (by volume) of acetonitrile and methanol that served as a mobile phase at a flow rate of 1.8 ml/min was first filtered (pore size diameter 0.45 μm) and then degassed in the VWR ultrasonic bath. Because of the maximal absorption of lycopene at 502 nm the latter wavelength was selected for the detection (Shimadzu SPD-M10Avp diode array detector).

The curve needed to calibrate the HPLC measurements was constructed (Fig. 2) by recording the chromatograms from a series of five standard solutions of lycopene covering the concentration range from 0.001 to 0.02 mg/ml. The concentrations of lycopene in the six selected tomato-based products were then deduced by comparing the areas of their chromatograms to those of standard solutions of lycopene (Fig. 2); the experimental conditions were the same as those used for the calibration procedure.

Once the lycopene concentrations of six tomato-based products have been determined, one was able to construct the calibration curve for the PA measurements. It is the availability of such a curve that eventually enables the quantification of lycopene in an unknown tomato-based product, without the need for a new HPLC measurement. A well defined measuring protocol was proposed and used consistently throughout all PA studies. With the tomato-based product loaded in a sample tray, the LED radiation was blocked for ten seconds to permit the PA signal on the lock-in amplifier to reach a

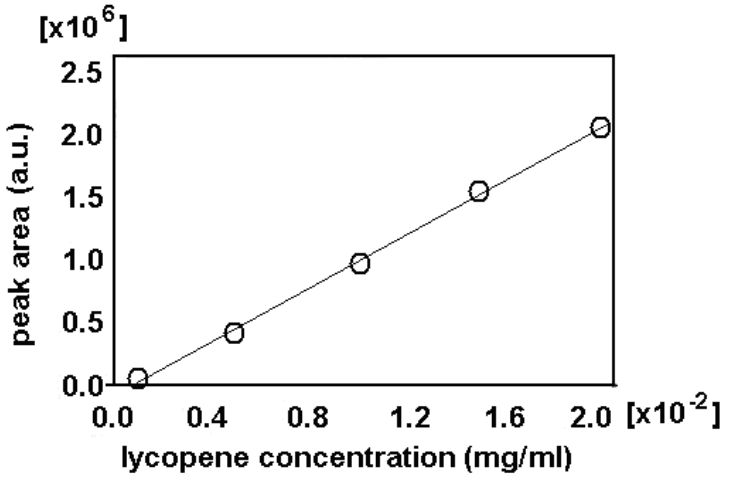

Fig. 2 The calibration curve for HPLC measurements obtained from the chromatograms from a series of five standard solutions. The concentration of lycopene in a solution varied from 0.001 to 0.02 mg/ml.

zero level. The beam was then unblocked and the PA signal allowed to stabilize during the next ten seconds. This instant was selected as the beginning of the actual measurement. Twenty successive PA signal readings were taken during 30 seconds (the integration time of the lock-in amplifier was 1 s) before the mean value has been calculated. The sample was then removed and PA cell opened and cleaned (ethanol and cotton swabs). Fresh quantity of the same tomato-based product was transferred into sample tray and the entire procedure described above repeated. The interval elapsed between the two successive measurements (it includes loading, proper cleaning and reloading of the PA cell) is of the order of only a few minutes. Overall, five measurements were performed with each tomato-based product before calculating the average and the corresponding standard deviation; the latter figure can be regarded as indicative for the analysis precision, i.e the repeatability achievable in multiple PA measurements.

3. Results

Figure 3 shows the PA signals obtained (at 25 Hz and with 4 mW incident LED power) from six samples plotted versus the concentration of lycopene (expressed in mg per 100 g fresh product weight) determined independently by the HPLC. Each data point in Fig. 3 represents the average of five PA measurements. Under the above specified experimental conditions the PA signal is linearly proportional (correlation coefficient R=0.97) to the concentration of lycopene found in selected tomato-based products.

The repeatability of the measurements expressed as a relative standard deviation of five independent measurements was 1.5 %). On the other hand, the instrumental precision based on the twenty consecutive readings of the PA signal from one and the same sample was better than 1%. The new approach proposed here for the quantification of lycopene in tomato-based products is not an absolute method, as it does not *directly* provides the concentration of this chromophore. Instead, the unknown concentration of lycopene in an arbitrary tomato-based product is deduced from the PA signal measured from the unknown sample and the calibration curve shown in Fig. 2.

For the proper interpretation of the measured data. the experimental conditions used during the actual measurement must be the same as those used for the calibration purposes; such a requirement is readily met in practice. An important advantage of the novel methodology proposed here is the fact that it obviates the need for the extraction of pigment (the samples are studied without any preparation, i.e. simply as they are) converting otherwise a tedious procedure into a much simpler approach.

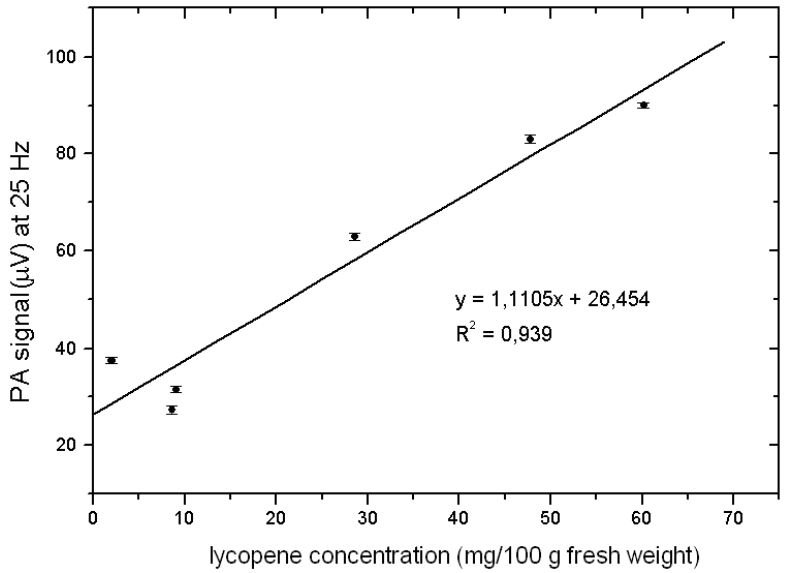

Fig. 3 The PA signal (at 25 Hz and 4 mW LED power) from six tomato-based products ploted as a function of lycopene concentration (expressed in mg/100 g fresh weight) determined independently by the HPLC method.

This in turn dramatically reduces the period needed to complete the analysis, and at the same time also pushes down expenses associated with the purchase of toxic chemicals. With this in mind, the new technique is expected to lead soon to the availability of a versatile, compact and affordable quality control tool for tomato industry processors.

References

[1] V.R. Reedy and R.R. Watson (Eds.): *Lycopene: Nutritional, Medicinal and Therapeutic Properties.* Science Publishers, London (2009)

[2] D.M. Barrett and G.E. Anthon: *Color quality of tomato products.* In: Color quality of fresh and processed foods. G.A. Culver and R.E. Wrolstad (Eds.). ACS Symposium. Series 983, Chapter 10, pp. 131-139 (2008)

[3] M. Baranska, W. Schutze and H. Schulz: *Determination of lycopene and beta carotene content in tomato fruits and related products: comparison of FT-Raman, ATR/FT-IR and NIR spectroscopy.* Analytical Chemistry. 78, 8456-8461 (2006)

[4] R. Choudray, T.J. Bowser, P. Weckler, N.O. Maness and W. McGlynn: *Rapid estimation of lycopene concentration in watermelon and tomato puree by fibre optic visible reflectance spectroscopy*. Postharvest Biology and Technology. 52, 103-109 (2009)

[5] D. Bicanic, V. Fogliano, S. Luterotti, J. Swarts, G. Piani and G. Graziani: *Quantification of lycopene in tomato products: comparing the performances of a newly proposed direct photothermal method and high-performance liquid chromatography*. Journal of Science of Food and Agriculture. 85, 1149-1153 (2005)

[6] D. Bicanic, R. Cuypers, S. Luterotti, M. Šporec, A. Zoppi and J. Vugec*: Practical, reliable and inexpensive assay of lycopene in tomato products based on the combined use of light emitting diode (LED) and the optothermal window*. Acta Chimica Slovenica. 55, 468-473 (2008)

[7] G. Sadler, J. Davis and D. Dezman: *Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates*. Journal of Food Science. 55, 1460-1461 (1990)