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Direct Quantification of Lycopene in Products Derived from Thermally Processed Tomatoes: Optothermal Window as a Selective, Sensitive, and Accurate Analytical Method without the Need for Preparatory Steps

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The concept of the optothermal window (OW) is proposed as a reliable analytical tool to rapidly determine the concentration of lycopene in a large variety of commercial tomato products in an extremely simple way (the determination is achieved without the need for pretreatment of the sample). The OW is a relative technique as the information is deduced from the calibration curve that relates the OW data (i.e., the product of the absorption coefficient β and the thermal diffusion length μ) with the lycopene concentration obtained from spectrophotometric measurements. The accuracy of the method has been ascertained with a high correlation coefficient ($R = 0.98$) between the OW data and results acquired from the same samples by means of the conventional extraction spectrophotometric method. The intrinsic precision of the OW method is quite high (better than 1%), whereas the repeatability of the determination (RSD = 0.4−9.5%, $n = 3-10$) is comparable to that of spectrophotometry.

Carotenoids, among the most abundant naturally occurring pigments in numerous commonly eaten fruits and vegetables,[1,2] are polyenic chromophores that impart yellow, orange, and red colors to plant tissues. The carotenoids that contain at least one β-ionone ring (they constitute about 10% of the more than 500 carotenoids known) show provitamin A activity. Most nutrition research studies have focused on six carotenoids (β-carotene, lycopene, α-carotene, lutein, zeaxanthin, and β-cryptoxanthin) found in relatively high concentrations in human blood. During the past decades carotenoid pigments have become highly publicized mainly due to their (i) potential role in cancer prevention,[2−4] (ii) antioxidant activity,[5,6] and (iii) ability to decrease the risk for coronary heart disease.[7] Carotenoids with nine or more conjugated double bonds are capable of quenching singlet oxygen[8] with lycopene being the most effective.[9]

Tomatoes and the products derived from thermally processed tomatoes are the major sources of lycopene (molecular formula $C_{40}H_{56}$) in the human diet.[10,11] It is believed that nowadays tomato and tomato products provide as much as 85% of dietary lycopene. The predominant geometrical isomer in fresh tomatoes is the all-trans lycopene. Processing and storage of tomato products are, on the other hand, responsible for the isomerization and autoxidation that cause a decrease of the total lycopene content and reduce the proportion of all-trans lycopene, affect the color, and contribute toward the development of grassy off-flavors.[12,13]

Reversed-phase HPLC, in either isocratic or gradient mode, is a powerful tool in the field of carotenoid research.[14−21] Tan[18] used gradient elution normal-phase open-column chromatography

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to separate carotenoids in tomato paste before analyzing them by reversed-phase isocratic elution HPLC. The principal carotenoid reported was lycopene followed by phytoene, β-carotene, and phytofluene. According to Thompson et al.,[19] the concentration of lycopene in tomato depends on the variety and its maturity at harvesting stage. Besides HPLC, conventional spectrophotometry in the visible is often used.[17,20−22]

Detection of carotenoids by both spectrophotometry and HPLC requires that the sample be presented as a clear liquid. This is because detectors used in both methods operate in the transmission mode. As the majority of products derived from tomatoes are actually optically thick and scattering semifluids, different approaches are presently used to extract the carotenoids prior to HPLC or spectrophotometric analysis. Unfortunately, such extractions are often laborious, time-consuming, expensive, and also prone to errors because of oxidation and/or losses during extraction. Rapid extraction of lycopene and β-carotene from tomato purée and from pink grapefruit homogenate was suggested by Sadler et al.[21]

The way to accelerate the analysis of carotenoids in tomato products is to develop an analytical method that relies on a different principle to measure absorbances in optically dense semifluids.

In principle, photothermal (PT) methods based upon conversion of absorbed optical energy into heat are potential candidates capable of substantially reducing the analysis time. Optical energy is absorbed by optically thick semifluids and eventually converted into thermal energy.[23] Although the initial absorption process is selective, it is common for excited electronic states in atoms or molecules to lose their excitation energy by a series of nonradiative transitions that results in a general heating of the sample. Unlike spectrophotometric detection, transparency is not a necessity for PT methods. The amount of generated heat proportional to the absorbance (and hence to the concentration of the absorbing analyte) is measured either directly or by observing changes in other parameters induced by the heat. Any constituent absorbing at the excitation wavelength will interfere with the analyte and hence produce additional heat.

Recently, the concept of the optothermal window (OW), one example among the PT methods, was applied in an initial attempt to determine lycopene in a few tomato purée concentrates.[22] The outcome of this preliminary investigation suggested the potential usability of the new OW concept in such studies but at the same time also revealed some shortcomings. The latter were primarily associated with the experimental setup and the mathematical model used to assess the product $\beta\mu$ (i.e., the dimensionless parameter derived from OW experiments); β is the absorption coefficient of the sample per unit length, while μ is the thermal diffusion length of the sample (at a given modulation frequency) that correlates positively with the concentration of the analyte.

This paper reports results upon the study conducted on 14 products derived from thermally processed tomatoes using the improved new OW cell. In choosing specimens for this study, the idea was to cover a wide range of lycopene concentrations. Therefore, test samples (bottled and canned) included juices, ketchups, passatas, purées, and pastes. All samples were studied without extraction or any other preparatory step. Finally, data obtained via OW experiments for specific products were compared to the results obtained from the same samples by means of extraction spectrophotometry. Data collected from all 14 samples enables us to construct a plot of $\beta\mu$ as a function of lycopene concentration. Such a plot serves as a "calibration curve" and can be used to rapidly quantify the content of lycopene in the unknown sample via a simple OW measurement.

EXPERIMENTAL SECTION

Samples, i.e., products derived from thermally processed tomatoes, included two tomato juices (A and B), two passatas (C and D), three ketchups (E, F, and G), two canned tomato purées (H and I), and five pastes (concentrated tomato purées) (J, K, L, M, and N) and were purchased in supermarkets in different countries.

Spectrophotometric Determination of Lycopene. All test samples were stored at +4 °C until the first analysis. Once the original can/tube was opened, the samples were kept at −18 °C. Before actual analysis the samples were equilibrated (overnight) at room temperature, homogenized on a shaker (for 3 h), and finally homogenized manually. The extraction procedure was modified from that reported by Sadler et al.[21] Each sample was weighed (2 ± 0.5 g) into a 100-ml Erlenmeyer flask, before adding 4 mL water and macerating for 1 min (magnetic stirrer). Then, 50 mL of a solvent mixture (hexane/acetone/abs EtOH, 2:1:1) was added and the sample was shaken for 10 min. After addition of 7.5 mL of water, the shaking continued for another 5 min. The layers separated, and the deeply colored hexane layer was diluted (10 to 100 times) with hexane. The spectrum of the fresh hexane solution containing lycopene and β-carotene was recorded, and the absorbance at 502 and 471 nm was measured after instrument recalibration. At 502 nm $\epsilon_{502} = 3150$ dL g^{-1} cm^{-1} for lycopene, whereas β-carotene was estimated from A_{471} with $\epsilon_{475} = 2049$ dL g^{-1} cm^{-1}.

After extraction, an almost colorless (beige to light orange) fluffy solid residue remained, whereas the polar layer was light yellow. All manipulations were performed under dim lighting conditions. Each product was analyzed at least in triplicate.

Determination of Lycopene by the Optothermal Window Method. The exploded view of the new, improved experimental setup for the OW studies is shown in Figure 1. It consists of the radiation source, a number of plane reflecting mirrors, the modulator, and a massive platform carrying the $x-y$ translation stage that accommodates the OW cell held in a gimbal mount. All these components were mounted on a granite table. The present construction enables the easy and precise alignment of the entire setup.

The 502-nm radiation was provided by a Lexel 851 CW argon ion laser (1). The laser beam reflected at two plane mirrors (2) was mechanically modulated (25 Hz) by means of a chopper (3)

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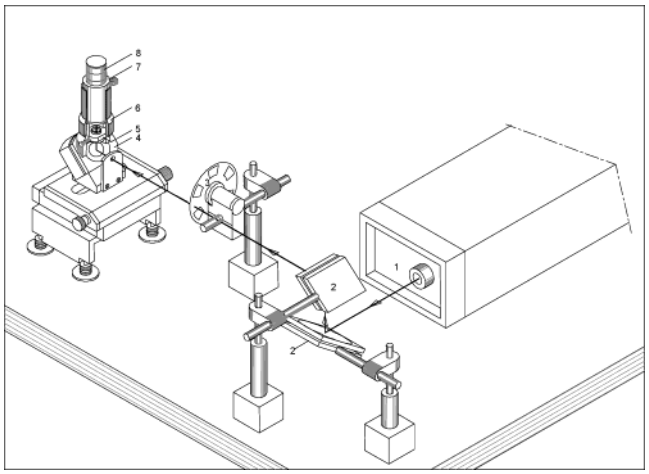

Figure 1. New experimental setup for the OW studies including a CW argon ion laser (1), a pair of two plane mirrors (2), a mechanical chopper (3), a plane mirror (4), a holder (5) that accommodates a disk and a piezoelectric ring, an adjustable sleeve (6), a housing (7), and the power detector (8).

manufactured by Stanford Research. The collimated beam was allowed to pass through a circular opening before striking a plane mirror (4) mounted at 45° in the polymer housing (7). As the centers of the opening and of the mirror are maintained at equal heights, the mirror (4) deflects the incoming radiation through a 90° angle. The OW cell was a 300-μm thick sapphire disk (diameter 14 mm; the transparency of the disk for 502-nm radiation is high) with a piezoelectric annular ring glued to its rear side. The entire unit was kept in holder (5) that in turn was mounted in the housing (7). The optical and symmetry axes of (5) and (7) coincided in space. The sleeve (6) served to shield the OW cell from undesirable effects due to external vibrations. In order to assess the amount of power reaching the sample in the OW experiment, the A501 Spectra Physics power detector (8) was mounted in the housing directly above the empty OW cell.

All OW experiments were performed under the same conditions ($\lambda_{exc} = 502$ nm and 25 Hz); the laser power at the site of the sample was typically 14 mW. At this level, no degradation of the samples was observed. A few grams of puree (or paste) or a drop of tomato juice (all are optically opaque samples) equilibrated (overnight) at room temperature were simply deposited directly on the central portion of the sapphire disk. The periodically modulated 502-nm radiation enters the disk from below and reaches the test sample on a disk's surface. Depending on the amount of lycopene in the specific tomato product, the latter absorbs the radiation as it penetrates through the sample. The generated heat diffuses back to the sapphire disk causing it to expand in a radial direction. Such a series of periodic expansions/contractions is sensed by the piezoelectric ring that produces periodic voltage (called optothermal signal) at the modulation frequency.

With the OW cell freshly loaded with a specific product, the magnitude of the optothermal signal was determined at 25 Hz by means of a Stanford Research SR830 lock-in detector using a 1 s integration time and a sampling rate of 18 consecutive readings within 20 s. After waiting for 30 s the same procedure was repeated twice (in total there were 54 readings). The standard deviation calculated from this kind of measurement provides information about the intrinsic precision of the OW method. The sample was then removed and the sapphire disk cleaned; the cleaning procedure is fast (on average 30 s) and extremely simple and involves only the use of wetted cotton swabs (initially by water and then by ethanol). The simplicity of both the sample presentation and the cleaning procedure allows for a large throughput. As sapphire is hard and resistant to cleaning agents, no deterioration in the performance of the OW detector was observed.

Then the disk was loaded with a new quantity of sample, and the sequence of steps described above was repeated. The same procedure was performed once again; overall, each tomato product was studied at least in triplicate. The standard deviation based on the outcome of such measurements gives an indication about the repeatability.

Solid Matter Content. Conventional gravimetry was used to determine the dry matter content in all 14 products equilibrated (overnight) at room temperature and homogenized (initially using a shaker for 2 h and then manually). Samples of 3 ± 0.5 g were weighed on an aluminum dish and dried (for 12 h) in a vacuum oven maintained at 75 °C and 40-mm Hg. At least three specimens of each product were taken for the analysis of the solid matter content.

RESULTS AND DISCUSSION

A very attractive feature of the OW method is the fact that the actual thickness L of the sample is of no relevance as long as it is larger than the inverse of the absorption coefficient per unit length, β, and the thermal diffusion length, μ, of the sample ($\mu < 1/\beta < L$). The thermal diffusion length μ depends on the thermal diffusivity α and the modulation frequency f as $\mu = (\alpha/\pi f)^{1/2}$; for a product specified by its α value, a low modulation frequency corresponds to the sampling of deeper-lying levels and vice versa. By virtue of the signal generation process mentioned above it is obvious that the amplitude of the OW signal is proportional to the amount of generated heat and thus to the concentration of the absorbing analyte. Only the heat generated within a layer one thermal diffusion length μ thick can effectively communicate with the sapphire disk and hence contribute to the OW signal. For example, at 25 Hz the thermal diffusion length of tomato ketchup is about 200 μm.

The OW signals obtained from a variety of samples were normalized to the signal acquired under identical conditions from a droplet of black Indian ink representing a very strong absorber.

Provided that a good thermal contact between the sample and the sapphire disk is secured and the requirement $\mu < 1/\beta < L$ is simultaneously met, the quantification of lycopene in optically opaque samples is possible as the theory predicts a correlation between the amplitude of the normalized OW signal and $\beta\mu$.[22]

The reader must be aware that it is $\beta\mu$ (in fact OW is a kind of combined spectroscopy and calorimetry) rather than β alone that correlates with the concentration of lycopene in the sample. Direct proportionality between the normalized OW signal and β can be established but only if the thermal diffusivity α of the sample is known (which is often not the case). However, it has recently been demonstrated that the thermal diffusivity and thermal effusivity of products derived from thermally processed tomatoes can be determined relatively simply and quickly by means of a photopyroelectric method.[23,24]

Table 1. Solid Matter, Lycopene, and β-Carotene in Thermally Processed Tomato Products[a]

sample code	solid matter (%)[b]	lycopene (mg g^{-1} dry matter)[b]	β-carotene (%)[c]
A	6.1 ± 0.0$_2$ (6)	2.13 ± 0.02 (6)	5
B	5.7 ± 0.1 (6)	1.60 ± 0.07 (3)	4
C	10.3 ± 0.1 (6)	2.00 ± 0.03 (6)	5
D	12.3 ± 0.1 (5)	1.80 ± 0.02 (3)	6
E	36.1 ± 0.1 (6)	0.43 ± 0.00$_2$ (6)	5
F	33.0 ± 0.1 (6)	0.33 ± 0.03 (6)	6
G	35.0 ± 0.1 (6)	0.35 ± 0.03 (3)	6
H	22.4 ± 0.1 (6)	1.82 ± 0.03 (6)	5
I	18.3 ± 0.1 (3)	1.05 ± 0.02 (3)	7
J	29.1 ± 0.2 (3)	1.78 ± 0.06 (6)	4
K	37.0 ± 0.2 (3)	1.30 ± 0.03 (6)	4
L	37.3 ± 0.1 (3)	1.63 ± 0.02 (9)	5
M	33.1 ± 0.1 (6)	1.26 ± 0.06 (6)	6
N	37.6 ± 0.1 (3)	1.47 ± 0.07 (9)	5

[a] Lycopene and β-carotene determined spectrophotometrically. [b] Mean ± SD value, number of independent analyses in parentheses. [c] Fraction of β-carotene in a mixture with lycopene.

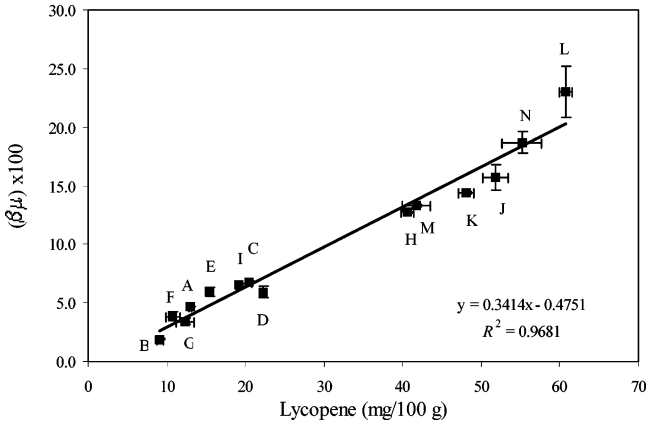

Figure 2. Correlation between the OW (n = 3−10) and spectrophotometric analyses (n = 3−9) for a number of commercially available products made from thermally processed tomatoes. Each point refers to the mean ± SD value.

The selective spectrophotometric determination of lycopene was achieved using the absorbance at 502 nm ($\epsilon_{502\ nm}$ = 3150 dL g^{-1} cm^{-1}. Table 1 shows the content of lycopene (expressed in mg per g of dry mass) and the standard deviation determined for various tomato products by means of spectrophotometry. For completeness, the β-carotene found in the test samples is reported as well. Also given is the dry matter content and the corresponding standard deviation.

Except for ketchups, the variability of the lycopene concentration per g of dry matter ranging from 1.1 to 2.1 mg g^{-1} gives information on the quality of the original tomatoes, cultivar, maturity when harvested, storage, and processing conditions.

Figure 2 shows 100$\beta\mu$ 100 obtained via the OW measurements plotted as a function of lycopene concentration determined by spectrophotometry for 14 products. The standard deviations are indicated along the two axes.

The "calibration curve" in Figure 2 indicates a high linear correlation (R = 0.98) between data obtained by OW and spectrophotometry from 14 different samples. The OW method is apparently also capable of measuring samples with lower lycopene content, such as tomato juices and ketchups.

Table 2. Precision Data

method (analysis)	RSD (%)[a]
OW (intrinsic)	<1 (54)[b]
OW (lycopene)	0.4−9.5 (3−10)
spectrophotometry (lycopene)	0.4−9.0 (3−12)
gravimetry (dry matter)	0.1−1.2 (3−6)

[a] Number of independent measurements or analyses in parentheses. [b] Modulating frequency: 25 Hz.

Data on the precision acquired in the OW, spectrophotometric, and gravimetric measurements in selected products derived from tomatoes are displayed in Table 2. The standard deviation characteristic for 54 consecutive OW measurements performed on one sample load was close to 1% for most products. However, the standard deviation based on multiple loadings is larger and reflects the inhomogeneity of the sample and the skill of the experimentalist. In addition to the common effect of increased imprecision at the limiting points of the linear range, it appears that tomato pastes rich in lycopene are less viscous than the other classes of samples studied here and establishing a good thermal contact with the sapphire disk (essential in OW measurements) might be more difficult.

The precision of both the OW and spectrophotometric analyses estimated based upon 3−12 independent analyses was the same and ranged between 0.4% and 10%. The highest imprecision was observed when analyzing lycopene in the richest samples and ketchups. It seems that imprecision of the OW analyses is mainly due to the inhomogeneity of the samples, whereas in spectrophotometric analyses multiple manipulations and the skill of experimentalist play a role as well.

The wavelength of choice in the OW experiment aimed at determining lycopene in products derived from tomatoes is 502 nm. Lycopene is by far the most dominant carotenoid in tomato products (as seen in Table 1, the fraction of β-carotene, the runner-up, is between 4% and 7%), and in addition, its absorption at 502 nm largely exceeds that of β-carotene. These facts support the statement that determination of lycopene at 502 nm is practically unaffected by the presence of β-carotene.

CONCLUSION

The performance of the newly proposed OW method for the rapid assessment of lycopene in 14 products derived from thermally processed tomatoes was evaluated by comparing $\beta\mu$ values acquired by the OW experiment and the concentration of lycopene in the same products determined by tedious and time-consuming classical spectrophotometry requiring extraction. A high linear correlation (R = 0.98) between the two methods was obtained confirming the acceptable level of accuracy in the OW analysis.

The OW method is not only extremely simple to perform but also selective and very precise (the precision of the determination is comparable to that of spectrophotometry). The most important advantage of OW is certainly the fact that tomato product specimens can be investigated directly, i.e., without any pretreat-

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ment. As it was demonstrated in this study, the choice of 502 nm as the measuring wavelength in the OW studies has enabled the determination of selective lycopene data in a large variety of commercial tomato products with lycopene concentrations ranging from 9 to 60 mg/(100 g). The OW method appears to be a major improvement over the regular analytical methods used to date.

The OW method is not an absolute one; it provides directly the values of the product $\beta\mu$. The concentration of lycopene is then deduced from a previously obtained calibration curve. Intimate thermal contact between the sample and the sapphire disk is a prerequisite. The "quality" of the thermal contact could not be quantified; for fluids and the pastes this issue is of less relevance as (with the exception of the less viscous samples) achieving a good thermal contact is rarely the problem. OW signals lower than expected (due to thermal loss mechanisms less heat is generated in the sample) provide experimental evidence for poor thermal contact. The results of this OW study suggest that the sensitivity of the present setup is sufficiently high to allow studies on products containing very low amounts of lycopene. This makes the OW approach potentially interesting for the measurement of lycopene in fruit juices, raw fruits, and other products.

Efforts are now in progress to explore the performance of the same OW concept in the case when inexpensive and compact radiation sources other than a laser are used for excitation. Studies aimed at investigating the potential of two other photothermal methods (photoacoustic and photopyroelectric techniques[25]) to detect lycopene in tomato products are already underway. Another point worth considering is the use of more than one wavelength in order to cancel out any "substrate" or interfering background. Such a dual wavelength modulation scheme could prove advantageous in OW as well as in photoacoustic and photopyroelectric measurements.

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