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Optothermistor as a Breakthrough in the Quantification of Lycopene Content of Thermally Processed Tomato-Based Foods: Verification versus Absorption Spectrophotometry and High-Performance Liquid Chromatography

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This study reports on the first use of the "optothermistor" as a novel, precise, fast, and low-cost detector of lycopene in a wide range of commercially available processed-tomato products. The quantitative performance of the new device was evaluated by comparing data obtained to that acquired by conventional methods, namely, absorption spectrophotometry and high-performance liquid chromatography (HPLC); the linear correlation was high ($R = 0.98$). The variation of data obtained with the optothermistor in a series of consecutive measurements performed with the same loading of the sample was better than 1%. However, the repeatability (RSD 0.5−9.0%, $n = 3-5$) achieved with the optothermistor by independent analyses (multiple loading) is comparable to that of HPLC and spectrophotometry. Results of the studies performed on the 19 products derived from tomatoes demonstrated that the optothermistor is suitable for selective, accurate, precise, and simple determination of lycopene (range = 7−75 mg/100 g of product weight) without the need for a sample pretreatment step. The estimated sensitivity of the present optothermistor is 2 mg of lycopene/100 g of product.

KEYWORDS: Optothermistor; lycopene; tomato-based products

INTRODUCTION

Lycopene ($C_{40}H_{56}$) is known for its capacity to inhibit the oxidation of low-density lipoprotein (LDL) cholesterol and DNA, reducing thereby the risk of the development of chronic diseases such as atherosclerosis, coronary heart disease, and certain types of cancer (*1*). This potent antioxidant and phytochemical nutrient is found in some fruits and vegetables. In particular, tomatoes are the major sources of lycopene in the human diet. Thermal processing of tomato is known to affect the bioavailability of lycopene, not its content. Other sources of lycopene include watermelon, guava, rosehips, and pink grapefruit (*2*).

The role of lycopene in human health led to a need for the assessment of the content of this antioxidant in commonly consumed foods. Different methods are currently used to reach this objective; however, inexpensive and rapid methodologies for the detection of lycopene are still lacking. The techniques most commonly used are elaborate as they require the sample to be present in the form of a clear liquid for the analysis. As a consequence, a time-consuming extraction is needed prior to the analysis by spectrophotometry and chromatographies (isocratic and gradient variants of HPLC and open column chromatography). Besides its thermolability, lycopene is also sensitive to light and oxygen; therefore, at each stage of the analytical procedure great care must be exercised to minimize the effects of the above-mentioned factors (*2*).

Throughout recent years, research efforts in our laboratory have concentrated on developing experimental methodologies that allow lycopene in tomato products to be determined directly, that is, without the need for extraction or other preparatory steps. Clearly, this requires the availability of new techniques the operation of which is based on principles that

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are entirely different from those normally used in conventional approaches. The most important requirement imposed on the candidate methods is their capability to rapidly quantify absorption in the strongly absorbing or completely opaque specimens.

The methods we are presently exploring in terms of their general potential to quantify absorbance in opaque media belong to the class of photothermal (PT) techniques (*3*). The latter rely on the detection of PT phenomena induced in the absorbing sample exposed to a periodically modulated radiation of selective wavelength. In the absence of radiative decay and photochemical reactions, the absorption of light by the material sample leads to the periodic generation of heat, which causes the corresponding changes of some physical properties either in the sample itself or in the contacting fluid. Examples of PT effects include the bulging of solid surface when heated, bulk deformation, generation of sound, production of refractive index gradient in the contacting fluid, and changes in the amount of thermal radiation emitted by the sample (*4*). These phenomena can be detected using a variety of PT methodologies such as photothermal beam deflection (PTBD), optothermal window (OW) spectroscopy, photoacoustic spectroscopy (PAS), dual-beam thermal lens spectrometry (TLS), and photothermal radiometry (PTR). Each PT technique makes use of a specific transducer that produces alternating voltage (termed PT signal) at the modulation frequency. The amplitude of the PT signal correlates positively with the amount of heat generated in the sample. Transducers used in various PT detection schemes include photodiodes (in PTBD and TLS), microphones (in PAS), and sensitive infrared detectors (in PTR) (*4*).

On the other hand, heat generated in the sample depends primarily on the concentration of the absorbing chromophore, the incident power reaching the sample, and the thermal properties of the sample under investigation. Direct proportionality between the PT signal and quantity of specific chromophore makes PT methods suitable for measurements of concentration. Indeed, in the past the PT methods have been successfully applied in the trace detection studies both in gaseous (*5*) and in liquid phases (*6*) as well in the investigation of strongly absorbing liquids (*3, 4*).

On the basis of the above-stated arguments it is clear that PT methods (when combined with the appropriate calibration procedure) are worth exploring in terms of their potential usefulness for the direct (no preparatory steps) and accurate quantification of lycopene in optically opaque samples such as tomato-based products. The first research study, the outcome of which indicated the intrinsic feasibility of the PT methods for direct detection of lycopene, described the application of the optothermal window (OW) concept to several tomato purée concentrates (*7*). Optimization of the experimental setup and improved design of the OW sensor enabled the extension of the measuring range of the OW technique to lower concentrations. Using the OW detector in conjunction with the 10 mW power for excitation provided by the argon laser, the content of lycopene in 20 different tomato products (used for direct consumption, as condiments and in food preparation) was accurately determined at 502 nm (*8*). The analytical performance of the OW method was compared to data obtained from the same samples by the conventional absorption spectrophotometry (*8*) and HPLC (*9*).

Although the concept of OW detection represents progress among existing methodologies for the quantification of lycopene in tomato products, this technique has an intrinsic drawback. In addition to the periodic vibrations resulting from the thermal waves generated in the absorbing sample, the piezoelectric material used as the actual transducer in OW detector is also sensitive to ambient vibrations. The latter are responsible for the production of parasitic PT signals that constitute a problem especially when the OW detector is operated in the acoustically noisy environments. Therefore, a method that is capable of quantifying absorption in opaque samples and at the same time virtually insensitive to external ambient external vibrations is preferred for use in realistic practice.

The study described in this paper reports the construction and first application of a new device, termed optothermistor, as a novel, practical, fast, specific, and low-cost detector of lycopene content (range from 7 to 75 mg/100 g of product) in a variety of products derived from tomatoes. The coined word "optothermistor" refers to a device consisting of a thin disk of sapphire (optically transparent and large thermal conductivity) and the ring-shaped thermistor. The thermistor, characterized by its negative temperature coefficient (NTC), is mounted to the rear of the disk (*10*).

A lycopene-containing sample placed atop the sapphire disk absorbs selectively the periodically modulated laser radiation, thereby generating heat and hence also thermal waves (a temperature field distribution). Such a periodic temperature rise/drop in the sample is sensed by the optothermistor and eventually converted to a voltage (PT signal) at the given modulation frequency.

Direct quantification of lycopene by means of the optothermistor was performed in 19 commercially available products made from thermally processed tomatoes. Despite their optical opacity, all samples could be studied simply as they are; that is, preparatory steps normally required to present a sample for analysis in the form of a clear liquid could be avoided. Comparison of data collected in optothermistor experiments and those obtained from the same 19 products by means of traditional methods (spectrophotometry in the visible range and HPLC) provided evidence about the feasibility of the newly proposed optothermistor. The speed of the measurements and the elimination of the extraction step significantly reduce the probability for isomerization to occur.

EXPERIMENTAL PROCEDURES

All samples investigated in this study were commercially available tomato products purchased from local supermarkets in different European countries. An attempt was made to obtain products from different brands; furthermore, none of the products investigated was older than the date of expiry reported on the label. The 19 samples analyzed in this study included 2 tomato juices (codes 1 and 2), 5 ketchups (codes 3−7), 2 passatas (codes 8 and 9), 3 tomato purées (codes 10−12], and 7 pastes (concentrated tomato purées) (codes 13−19). Mainly for economic reasons 14 of these samples were studied by spectrophotometry and the remaining 5 by HPLC.

Determination of Lycopene by Means of Optothermistor. Figure 1 is the exploded view of the homemade apparatus used in this study. The radiation emitted by a 20 W halogen lamp (A) is electronically modulated at 8.3 Hz; this frequency is close to the upper limit imposed on the operation of both the halogen lamp and the optothermistor itself, as explained below. Schott BG18 colored glass (B) with a central wavelength of 500 nm and a 40 nm bandwidth was used to filter the radiation of the halogen lamp before it was focused by lens (C) on a polymer optical fiber (E), 1 mm in diameter. The reason for selecting filter B with the aforementioned spectral characteristics is that its central wavelength (500 nm) is very close to 502 nm, corresponding to one of the absorption peaks of lycopene. The small beam divider (G) made of glass directs a fraction of emitted radiation to the photodiode (D) used to regulate the current of the lamp and hence its output power.

The unit (F) accommodates the optical fiber and the optothermistor that consists essentially of a 130 μm thick sapphire (known for its

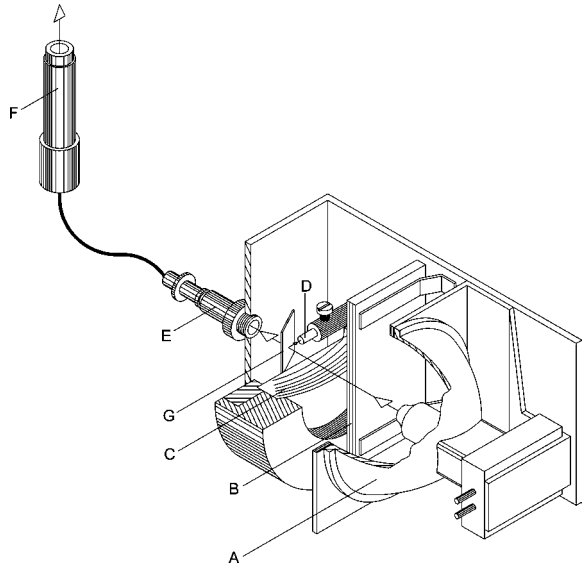

Figure 1. Exploded view of apparatus used to quantify lycopene in products derived from the thermally processed tomatoes: halogen lamp (A), band-pass filter (B), converging lens (C), photodiode (D), optical fiber (E), unit accommodating optothermistor (F), and beam divider (G).

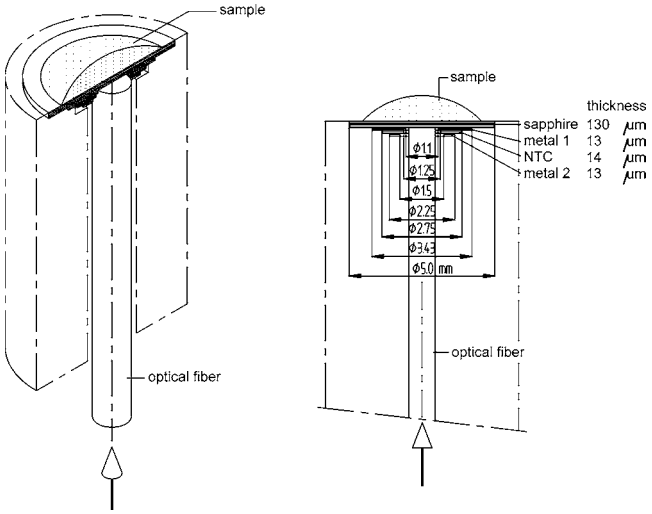

Figure 2. Exploded view (left) and transversal cross section (right) of the optothermistor used in this study. The arrow indicates the direction of the incoming (filtered) radiation beam.

superior optical, mechanical, and chemical properties) disk 5 mm in diameter. Attached to the rear of the sapphire disk is a thermistor; this is actually the annular NTC ring (diameter = 2.57 mm, thickness = 14 μm), each side of which is provided with a deposited thin (13 μm) metallic layer (diameter = 3.43 and 2.25 mm). **Figure 2** shows the exploded view and the transversal cross section of the optothermistor: the sapphire disk, the NTC with two metalled layers, and the optical fiber are all integrated in a single unit, as stated above. The fiber is placed directly against the rear face of the sapphire disk. Except for its central section (defined by the circular area 1.5 mm in diameter) the sapphire disk is coated to minimize the effect of undesirable reflections.

In the aforementioned optothermistor the NTC is used to measure the variations of temperature. However, the sensitivity (current−voltage characteristics) of the NTC itself depends on the actual operating temperature. This effect was accounted for by using the specially designed preamplifier with the resistance of the compensation resistor selected such as to match that of the NTC at room temperature. In such a manner potential problems associated with the varying sensitivity of the detector caused by changes of mean temperature are eliminated.

As the sapphire disk is actually "open" (i.e., not enclosed in a chamber), its surface is easily accessible so that loading/removal of the sample can be performed quickly and in an extremely simple manner. Likewise, it is relatively simple to shield the optothermistor from external electric fields and from scattered light. A small quantity (<0.2 mL) of homogenized (mixer) tomato product is deposited directly on a clean upper surface of the sapphire disk. Filtered radiation from the halogen lamp propagates through the optical fiber and eventually enters the sapphire disk from below. It then passes through the same disk practically without any loss (sapphire is transparent at these wavelengths) and ultimately reaches the tomato product. The sole fact that the incident radiation, unlike in traditional spectroscopic methods, enters the optically opaque sample (tomato product) from below (i.e., through the sapphire disk) rather than from above is essential for the operation of the optothermistor when used as the detector of absorbance. Furthermore, the actual quantity of the tomato product deposited on the surface of the sapphire disk is of no relevance as long as the sample under investigation covers the central portion of the sapphire disk illuminated by the incoming radiation beam (*8*).

Heat generated in the sample on account of absorption diffuses from the heated spot through the sapphire disk to the thermistor. The distance across which the heat diffuses must be comparable to the diameter of the incident radiation beam. This requires (i) the illuminated region of the sample to be small and (ii) a long thermal diffusion length, $\mu = (\alpha/\pi f)^{1/2}$, in the sapphire disk ($\alpha$ is the thermal diffusivity and f is the modulation frequency) and, consequently, low modulation frequency.

At high f values μ is short, resulting in a less significant diffusion of heat to NTC, leading to a weak signal and a poor signal-to-noise ratio. The sensitivity of the detector used in this study begins to decrease for modulation frequencies exceeding 10 Hz. At selected operational frequency (8.3 Hz) the thermal diffusion length in sapphire is 706 μm, which is comparable to the distance between the heated spot and the thermistor. Due to the small diameter (1 mm) of the thermistor (mounted close to the illuminated region), there is enough time for heat to diffuse between consecutive modulation cycles; consequently, the volume of the tomato product being probed is small. The corresponding periodic variations of temperature are finally sensed by the thermistor (outside the illuminated area) and transformed into electrical voltage termed the PT signal. Given the modulation frequency f, the magnitude of the PT signal is directly proportional to the product $\beta\mu$. (*7*) The parameter (β) is the chromophore's optical absorption coefficient per unit length (at a given wavelength) defined as the product of molar absorptivity (ϵ) and molar concentration (c) of chromophore. The two-phase lock-in amplifier measured the PT signal. The proportionality between the magnitude of the PT signal and the product $\beta\mu$ (or $\epsilon c\mu$) for a specific tomato product clearly indicates that both optical spectroscopy and calorimetry play a role in the newly proposed optothermistor detection. For appropriate quantitative interpretation of data collected in opto-thermistor measurement, the obtained PT signals must be properly normalized. This was accomplished by dividing them by the lock-in signals acquired under identical experimental conditions from a strongly and uniformly absorbing sample such as black Indian drawing ink (*11*). Specially designed software computes the normalized signals and converts them to the dimensionless product $\beta\mu$; these values are read directly from the instrument display.

The protocol that was consistently maintained during optothermistor measurements was as follows. A specific tomato product (liquid or paste) on a sapphire disk was irradiated at 8.3 Hz for 20 s while at the same time measuring the PT signals (amplitude and phase) and calculating $\beta\mu$ values. Because it always takes some time for the sample to reach thermal equilibrium, data collected during the first 5 s were rejected. The amplitude (and phase) of PT signals acquired during the remaining 15 s was then automatically averaged and the mean $\beta\mu$ value displayed. After a delay of ~30 s, the same sequence was repeated; overall, five such series were performed. The standard deviation achieved for $\beta\mu$ by optothermistor measurements performed on such a "single-load" sample was better than 1% of the measured value. Next, the sample was removed from the sapphire disk and the surface of the sapphire cleaned with cotton swabs dipped in water and ethanol. Then, a fresh quantity of the same tomato product was deposited on the sapphire plate and the aforementioned cycle run again. Depending on a specific product, three to five repetitive series of measurements on "multiload" samples have been performed. In this manner, between

15 and 25 values for $\beta\mu$ product were eventually available for each of the investigated tomato products. All products were investigated in the same manner. The simplicity of both sample presentation and the cleaning procedure allow for a reasonable sample throughput.

It is important to note that by virtue of the operational principle of the optothermistor, the magnitude of the generated PT signal is due to all constituents that absorb at of the analytical wavelengths; this situation is equivalent to the spectrophotometric measurement of the whole extract. In tomatoes and tomato products in which lycopene is a dominating component, the optothemistor will therefore give directly a level of this principal carotenoid.

Spectrophotometric Determination of Lycopene. The samples were maintained sealed in the refrigerator (at 4 °C) until the actual analysis; once opened, the products were stored at −18 °C. Prior to the analysis, the samples were equilibrated overnight at room temperature and homogenized [initially by means of a shaker (3 h) and then manually]. Each sample was weighed (2 ± 0.5 g) into a 100 mL Erlenmeyer flask. Double- and triple-concentrated tomato products were reconstituted with 4 mL of water to achieve complete and reproducible extraction; the mixture thus obtained was homogenized for a minute using a magnetic stirrer. Upon addition of 50 mL of mixture (hexane/acetone/absolute EtOH in proportion 2:1:1 by volume) the sample was shaken during 10 min (*12*). Then 7.5 mL of water was added and the shaking continued for another 5 min. A deep orange hexane layer separated from a light yellow polar phase. What is left after the extraction step is an almost colorless (beige to light orange) fluffy solid residue. The hexane extract was then diluted (10−100 times) with hexane and the spectrum of the fresh solution recorded after recalibration of the UV−vis spectrophotometer (Agilent 8453 run by PC-HP 845x system). The amount of lycopene in the specific product was estimated from the value of absorbance at 502 nm ($\epsilon_{502} = 3150$ dL g^{-1} cm^{-1}). All sample manipulations were performed under "from-light-protected" conditions; at least three independent analyses of each product have been completed.

Determination of Lycopene by HPLC. The procedure originally proposed by Tonucci et al. (*13*) was modified and used here. The extraction of carotenoids was accomplished with THF in the presence of 0.01% butylated hydroxytoluene (BHT). Dry extract obtained in the flow of nitrogen in dark tubes was resuspended in 5 mL of chloroform. This implied a negligible reduction of carotenoid (recovery of ethyl-β-apo-8′-carotenoate that served as a standard was estimated to be 98%) but significantly improved reproducibility.

A Shimadzu LC 10 HPLC equipped with a diode array detector and a Supelcosil C_{18} column (250 × 4.6 mm; 5 μm particle size, 100 Å) was used to analyze the samples. Unlike in ref *13*, the mobile phase was prepared from acetonitrile (solvent A) and the mixture of methanol, hexane, and methylene chloride (1:1:1, by volume, solvent B). The separation was achieved at a constant flow rate (0.8 mL min^{-1}) using the linear gradient conditions, that is, A/B = 82%:18% (at $t = 0$), 76%:24% (at $t = 20$ min), 58%:42% (at $t = 30$ min), 39%:61% (at $t = 40$ min), and back to initial condition again (at $t = 45$ min). Such a judicious choice of phase and gradient resulted in a better separation of peaks. The quantification of carotenoids was achieved using Class M10-A Shimadzu software and the calibration curve constructed with the commercially available TLC-purified lycopene. The solutions in hexane were prepared on the basis of $\epsilon_{471} = 3450$ dL g^{-1} cm^{-1} (for lycopene). The extraction was repeated twice in a duplicate analysis each time; hence, four results have been obtained for each sample. Values obtained by HPLC refer to the sum of all lycopene isomers; usually in the products made from tomatoes *all-trans*-lycopene constitutes >95% of the total lycopene content.

RESULTS AND DISCUSSION

The concentrations, expressed in milligrams per 100 g of product (wet basis), of lycopene in various tomato products found by spectrophotometry and HPLC were used to construct the horizontal axis in **Figure 3**; the 100-fold values for $\beta\mu$ (obtained directly from optothermistor measurements) are plotted along the vertical axis. Bearing in mind a large variety of tomato products, the linear correlation ($R = 0.98$) between data obtained by the optothermistor and the two classical methods is surprisingly high. The intrinsic precision (defined as the standard deviation based on five consecutive measurements performed with the optothermistor using a single sample load) ranges from 0 to 3.8%; for most products it was <1%. However, the standard deviation (also shown in **Figure 3**) deduced from optothermistor data obtained during repeated measurements (multiload sample) is larger as it reflects the cumulative effect of the sample's inhomogeneity, signal noise, the skill of the experimentalist, etc. The repeatability was found to be independent of the specific product class. The precision (RSD = 0.5−9.0%, $n = 3$−5) attained in optothermistor analyses is comparable to that of HPLC (RSD 0.2−8.5%, $n = 2$) and spectrophotometry (0.4−9.0%, $n = 3$−9).

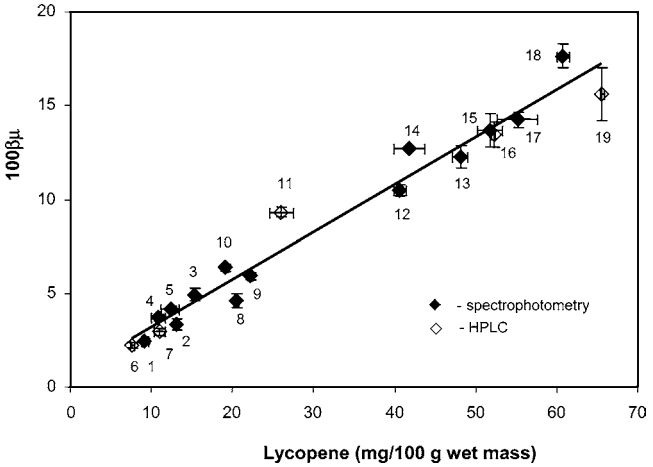

Figure 3. Correlation found between the optothermistor analyses (n = 3–5) and spectrophotometric (n = 3–9) and HPLC analyses ($n = 2$) of commercially available products derived from the thermally processed tomatoes. The data shown are averages with ±SD bars. The regression line reads $y = 0.2516x + 0.7138$; $R^2 = 0.9653$.

It appears that the precision and the sensitivity achieved here with the compact instrument consisting of the optothermistor and the halogen lamp are comparable to those obtained from the same samples when using a much larger argon ion laser (502 nm) for excitation and the optothermal window as a detector instead. The selection of 502 nm laser radiation as the analytical wavelength was governed primarily by the fact that at this wavelength the spectral contrast between lycopene and interfering β-carotene is maximal. Bearing in mind that lycopene in tomato products dominates above β-carotene ensures both high specificity and good sensitivity of optothermistor.

In the optothermistor experiment an attempt was made to direct ∼40 mW of filtered polychromatic radiation (wavelengths ranging between 480 and 520 nm) from the 20 W halogen lamp into an optical fiber 1 mm in diameter. If all radiation would have indeed been focused to a spot of that size, the expected power density at the entrance of the fiber would be ∼50 kW/m^2. This is considerably higher than the intensity levels normally used for in vivo studies of photochemically sensitive samples. In our optothermistor experiment performed with intermittent light (modulation frequency = 8.3 Hz) neither degradation of PT signal nor visible damage to tomato products has been observed in the course of the study. The same conclusion could be drawn from OW studies (*8*) performed on the same samples using the collimated beam (10 mW, effective diameter = 2 mm) of monochromatic argon ion laser radiation (502 nm).

To summarize, the outcome of the optothermistor study conducted on 19 tomato-based products shows that selective,

accurate, and direct (no need for pretreatment step) determination of lycopene can be accomplished. At 8.3 Hz the thermal diffusion length is sufficiently large to enable the interrogation of characteristic samples' volume (on the order of tens of a milliliter). The values of $\beta\mu$ (proportional to the normalized signals) obtained for different products directly from optothermistor measurements correlate ($R = 0.98$) positively with the content of lycopene (determined by spectrophotometric and HPLC methods) across a wide concentration range. With the present setup it was possible to accurately determine the content of lycopene in tomato products; the concentration of this compound ranged from 7 to 75 mg/100 g of wet product. Overall, the variation between individual measurements was <1% of the measured value. On the basis of the signal-to-noise ratio of the PT signal obtained at 8.3 Hz from tomato juice containing 7 mg of lycopene/100 g product, one arrives at 2 mg of lycopene/100 g of product as the detection limit for the present optothermistor device. This might allow lycopene measurements by optothermistor on fresh tomatoes and grapefruit juice; this research as well as detection of lycopene in tomato powders is currently in progress.

The optothermistor method yields directly the level of principal carotenoid in tomato products in which a single carotenoid (here lycopene) dominates. At the same time this is also the limitation of the optothermistor when compared to the HPLC method capable of providing the concentration of each carotenoid separately. The elimination of the extraction step greatly reduces the probability for degradation/oxidation during the analysis. Unlike microphone and piezoelectric crystals that are normally used as transducers in photoacoustic and optothermal detectors, the optothermistor is virtually insensitive to ambient vibrations, which enables this device to be used in acoustically noisy environments.

For reproducible quantitative measurements, thermal contact between the sample and sapphire disk is essential; furthermore, the experimental geometry, the modulation frequency, and the actual power entering the test sample must be the same for all samples. Homogenization of the sample is rather important as the presence of air bubbles and/or other inhomogeneities in the sample could (via internal reflections) influence the thermal transport.

In conclusion, this paper reports on the results of initial measurements the objective of which was only to demonstrate the applicability of the optothermistor detector in the analysis of the tomato products containing lycopene. As stated above, the analytical signals for lycopene measured by the optothermistor correlate with the well-established methods such as spectrophotometry and the HPLC. In the following research phase efforts will be made to (i) fully validate the optothermistor as a new analytical tool and (ii) quantify lycopene in tomato products by using data provided solely by the optothermistor itself.

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