Rapid, accurate, and direct determination of total lycopene content in tomato paste

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Lycopene that imparts red color to the tomato fruit is the most potent antioxidant among carotenes, an important nutrient and also used as a color ingredient in many food formulations. Since cooked and processed foods derived from tomatoes were shown to provide optimal lycopene boost, products such as paste, puree, juice, etc. are nowadays gaining popularity as dietary sources. The analysis of lycopene in tomato paste (partially dehydrated product prepared by vacuum concentrating tomato juice) is carried out using either high pressure liquid chromatography (HPLC), spectrophotometry, or by evaluating the color. The instability of lycopene during processes of extraction, etc., handling, and disposal of organic solvents makes the preparation of a sample for the analysis a delicate task. Despite a recognized need for accurate and rapid assessment of lycopene in tomato products no such method is available at present. The study described here focuses on a *direct* determination of a total lycopene content in different tomato pastes by means of the laser optothermal window (LOW) method at 502 nm. The concentration of lycopene in tomato paste ranged between 25 and 150 mg per 100 g product; the results are in excellent agreement with those obtained by spectrophotometry. The time needed to complete LOW analysis is very short, so that decomposition of pigment and the formation of artifacts are minimized. Preliminary results indicate a good degree of reproducibility making the LOW method suitable for routine assays of lycopene content in tomato paste. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1512774]

I. INTRODUCTION

Fat-soluble lycopene ($C_{40}H_{56}$, molecular weight 536.85), the most unsaturated (11 conjugated double bonds) acyclic carotenoid is synthesized by plants and microorganisms. Found in fruit and vegetables this pigment acts as an antioxidant and scavenger of free radicals.[1] Results of epidemiological studies show that the intake of lycopene rich foods is associated with better protection against some cancers and lowered risk of developing certain types of diseases.[2] Since the human body cannot manufacture lycopene, this nutrient must be supplied externally in a diet. Lycopene is exceptionally abundant in ripe tomato (typically 5 mg per 100 g raw tomato; in some varieties more than 40 mg per 100 g) comprising as much as 85% of all the pigments present.[3] Lycopene and other tomato solids (sugars, organic acids, pectin, minerals, and salt) typically together constitute 6%–8% of the total weight.

A large portion (80%) of all tomatoes produced is consumed in the form of processed products.[4] It was shown that thermally processed tomato products have a higher content of lycopene making it also easily absorbable by the body.[5] One among frequently consumed products derived from tomatoes is tomato paste (it contains between 8% and 38% tomato solids).

Both physical and chemical methods are used to determine lycopene content in tomato-based foods.[6] Physical methods study the relationship between color parameters and the concentration of lycopene, while chemical techniques focus on the quantification of lycopene extracted from the tomato tissue. Nowadays, the best approach towards the analy-

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sis and identification of lycopene is that of high pressure liquid chromatography (HPLC); recently the latter method when combined with a thermal lens spectrometry was demonstrated to be highly sensitive in detecting several carotenes in edible oils.[7] In addition to HPLC, traditional spectrophotometry is often used to estimate lycopene in products derived from tomatoes. Both HPLC and spectrophotometry are time-consuming and require substantial precautionary measures to avoid artifacts and quantitative losses due to the instability of lycopene. The food processing industry therefore prefers novel techniques capable of quantifying lycopene in tomato pastes directly, i.e., without a need for extraction.

In a study described in this article, the laser optothermal window (LOW) (at 502 nm) was used for the first time to quantify total concentration of lycopene (all-*trans* lycopene and most of *cis* lycopene) in tomato pastes containing varying amounts of dry matter. The generation of OW signals in such optically opaque semifluids (not amenable for analysis by traditional methods) was discussed elsewhere.[8] Data acquired by the LOW method was compared to spectrophotometric results obtained from the same samples.

II. EXPERIMENT

Six tomato pastes (for simplicity termed here as 1, 2, 3, 4, 5, and 6) with a varying content of dry matter were purchased in supermarkets in different countries. The selection included concentrated (purees 1 and 4), double concentrated (2 and 5) and triple concentrated (3 and 6) tomato pastes. Lycopene absorbs[9] strongly in the visible (peaks at 444, 470, and 502 nm) and less strong in the IR (CH out of plane deformation at 966 cm^{-1}). Since at 502 nm the interference of other carotenoids is minimized all measurements were performed at this wavelength. The 502 nm radiation was provided by a cw argon ion laser Lexel 85; the actual power reaching the sample was 6 mW. The LOW device is a sapphire disk (300 μm thick and 20 mm in diameter) with the annular ring (made of lead-zirconate-titanate, PZT) bonded to its bottom. Another ring made of a glass (15 mm inner diameter, height 6 mm) was glued to the sapphire's top face to define sampling volume. After squeezing it out of the tube, the paste was homogenized (hand mixing) before transferring it to OW cell to fill the sampling volume (typically 1 cm^3) completely. An iris diaphragm was used to shield the PZT detector from undesirable heating caused by forward propagating laser radiation.

The dry matter content of tomato pastes was determined by weighing (in triplicate) approximately (5.00 ± 0.05) g in alumnium dishes and drying in a vacuum over (75 °C) for 12 h. The dry matter content (expressed in percent per mass) was 18.05, 30.28, 37.38, 23.55, 29.78, and 37.93 for tomato pastes 1, 2, 3, 4, 5, and 6, respectively.

Calibrating the response of the LOW setup requires the knowledge about the amount of lycopene in tomato pastes. Three pastes (1, 2, and 3) were arbitrarily chosen for calibration purposes and lycopene concentration determined by means of spectrophotometry[10] at 502 nm; measurements were carried out in triplicate. The amount of lycopene found per 100 g paste was (32.10 ± 5.34) mg, (85.19 ± 9.78) mg, and (146.02 ± 19.74) mg in tomato pastes 1, 2, and 3, respectively.

III. RESULTS AND DISCUSSION

To make sure that at 502 nm no other components but lycopene contribute significantly to the LOW signal, an initial study was performed using pastes all characterized by the same dry matter content. This was achieved by adding a controlled quantity of distilled water to paste (dry matter content originally present in each paste is precisely known) to achieve a dry matter target value of 16%. The signals obtained from these diluted pastes were comparable (within 2%) as expected.

In the next study one has investigated tomato pastes without any preparation, i.e., just as they are when squeezed out of the tube. The lock-in signals (at 1 Hz) were recorded for all pastes (each sample measured in triplicate); 500 successive lock-in readings during 15 s were taken and averages calculated. These values were then corrected for background absorption (OW cell loaded with water) and normalized to those obtained under identical experimental conditions from the black ink (β is 1.1×10^4 cm^{-1} at 502 nm[11]) acting as a strongly absorbing reference. The magnitude Ψ of the thus obtained normalized OW signal is given by[11]

$$\Psi=\beta_p\mu_p[(1+\beta_p\mu_p)^2+1]^{-1/2}(1+e_p/e_w)^{-1},$$

where e_p (varies from 1360 to 1490 $W/s^{1/2}/m^2\,K$ depending on a dry matter content) and $e_w=9900$ $W/s^{1/2}/m^2\,K$ are thermal effusitivities of the paste[12] and of the sapphire window, respectively. Symbols β_p and μ_p are tomato paste's absorption coefficient per unit length (at a given wavelength) and thermal diffusion length (at a given modulation frequency). As an example, the lock-in signals (at 1 Hz) obtained at 502 nm from a triple concentrated paste (sample 3), water, and the black ink were (137.8 ± 0.2) μV, (2.7 ± 0.5) μV, and (159.6 ± 0.1) μV. With 13.1×10^{-4} cm^2/s as thermal diffusivity [13] (this gives $\mu_p=2.02\times10^{-3}$ cm at 1 Hz) of tomato paste 3, one obtains $\Psi=0.979\pm0.003$; likewise, $\Psi=0.738\pm0.004$ and 0.378 ± 0.007 for pastes 2 and 1. As to the accuracy of Ψ achieved in the repeated measurement, the typical error was about 4%.

The knowledge of lycopene concentration c in tomato pastes 1, 2, and 3 and the corresponding Ψ values (obtained via the LOW experiment) allow for the construction of the calibration curve shown in Fig. 1. Data plotted is the average of three consecutive measurements, the extent of uncertainty for both parameters is specified. The best fit ($R^2=0.9779$) equation reads $\Psi=0.0053c+0.2323$ with c being expressed in mg/100 g paste. Direct heating of the PZT detector is most likely the reason for the nonzero intercept of the Y axis observed in Fig. 1.

In a final stage of this study, three remaining pastes (4, 5, and 6) were investigated using the same LOW setup and their lycopene content estimated from experimentally obtained Ψ values and the plot in Fig. 1. For pastes 4, 5, and 6 this gives $\Psi=0.566\pm0.023$, 0.649 ± 0.026, and 0.716

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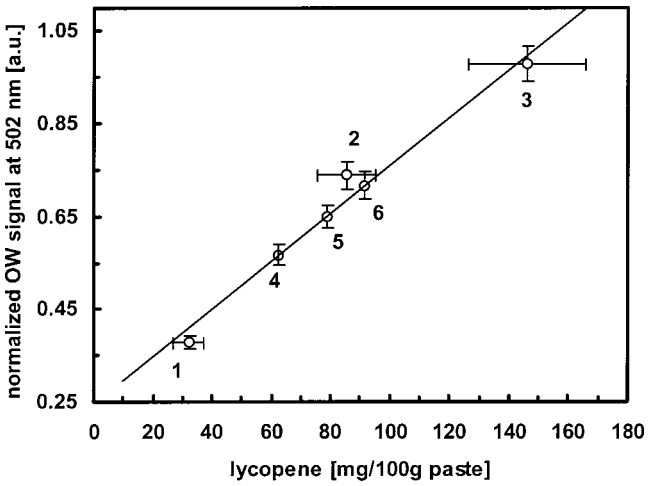

FIG. 1. Magnitude of the normalized OW signal Ψ (obtained at 502 nm via LOW measurement at 1 Hz) plotted vs concentration of lycopene per 100 g product [determined at 502 nm via conventional spectrophotometry (Ref. 10)]. The numbers 1–6 correspond to different tomato pastes used as test samples.

± 0.029, respectively. The concentrations of lycopene corresponding to mean values of Ψ for samples 4, 5, and 6 are 61.08, 78.62, and 91.26 mg per 100 g paste. Finally, the concentration of lycopene in pastes 4, 5, and 6 was also measured by the spectrophotometric method mentioned above. The amount of lycopene found in samples 4, 5, and 6 is (62.23 ± 4.15) mg, (88.88 ± 6.03) mg, and (92.36 ± 13.93) mg per 100 g paste.

IV. CONCLUSION

The LOW method at 502 nm was used for the first time to quantify lycopene in several tomato pastes. Unlike other techniques that all require extraction of lycopene from tomato tissue before it can actually be quantified, the LOW approach was shown to be capable of providing accurate and outstandingly reproducible results directly, i.e., without any sample preparation and using only minute quantities of the sample. Initial results obtained by LOW agree very well with those acquired by spectrophotometry. Study of a considerably larger number of pastes differing in a content of dry matter, texture, and reflection property is currently underway. Furthermore, there are considerable ongoing research efforts, the objective of which is to optimize experimental conditions (modulation frequency, incident laser power, choice of a disk material, homogeneity, elimination of scattered radiation, etc.). Eventually, the LOW method used here could evolve into a very practical, rapid, and sensitive technique for (i) routine estimation of lycopene content in pastes and other products derived from tomatoes, (ii) testing of functional foods and pharmaceutical grade products, and (iii) the bioavailability studies[14] and a wide range of other applications in the food industry.

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