Potential and Limitations for Determining Lycopene in Tomatoes by Optical Methods

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Keywords: lycopene, tomato, color

Abstract

Lycopene in tomatoes and tomato products is routinely determined by extraction into organic solvents and spectrophotometric quantification. The direct determination of lycopene, by measuring the color or other optical properties of a tomato homogenate, would be an attractive alternative. We have evaluated two instruments for this purpose. The color of tomatoes and tomato products is routinely determined with a reflectance colorimeter such as a LabScan (Hunter Lab). We have examined whether the parameters determined with this instrument correlate sufficiently well with the lycopene content of raw tomato juice to allow for the prediction of lycopene levels. CIE "a" values were not linearly related to lycopene content but could be fit to a logarithmic regression line. A better, but still logarithmic, fit was obtained when the parameter optical density at 560 nm minus the optical density at 700 nm, was plotted versus lycopene. The absorbance of light transmitted through tomato juice was also measured with a Hunter Lab UltraScan. The parameter, absorbance at 560 nm minus absorbance at 700 nm was linearly related to the lycopene content of the tomato juice. The slope of the regression line, however, was affected by the method used to homogenize the tomato. Measured values for (A560-A700) were also substantially lower in cooked versus raw tomato juice samples that contained equal amounts of lycopene. Evidently, factors other than the total lycopene content greatly affect the absorbance values measured by the UltraScan.

INTRODUCTION

In tomatoes, lycopene is the principal carotenoid, comprising about 95% of the total, and is responsible for the red tomato color. Intuitively it seems apparent that the intensity of tomato color should correlate with the total lycopene content. Correlations between color, usually reported as CIE **a** value, and lycopene content have been reported and proposed as a way to estimate the lycopene content (Arias et al., 2000; D'Souza et al., 1992). In addition, direct measurements of light absorbance by tomato juice, at the wavelengths of maximum absorbance by lycopene, have also been shown to correlate with lycopene content. This also has been proposed as an alternative method for estimating lycopene in tomatoes (Davis et al., 2003). Here we have evaluated these methods and examined some of the factors that may affect their usefulness.

MATERIALS AND METHODS

Tomatoes used to prepare fresh tomato homogenates were both processing types (evaluated in annual variety evaluation program; specific varieties not specified) and fresh market type ('Early Girl'). Homogenates were prepared either by grinding in a blender with added water (in some cases at a 1:1 ratio of water to tomato, in others at a 3:1 ratio), or by grinding in a mortar and pestle followed by passing through a screen to remove any large pieces of skin. Various dilutions of these homogenates were then examined. When homogenized in a blender, samples were allowed to stand for one hour prior to measuring to allow any air bubbles to completely dissipate. Microwave hot-break juice from tomatoes was prepared and stored frozen as described (Barrett and Anthon, 2001). For

optical measurements 5 g of juice was diluted to 20 mL with water.

Absorbance measurements of the tomato homogenates and hot-break juice samples were made with an UltraScan XE (Hunter Associates Laboratory, Reston, VA), as described by Davis et al. (2003). Measurements were made in transmittance mode using a 20 mL, 1 cm path-length cuvette. The absorbance difference between 700 nm and 560 nm was then determined. The same samples were also analyzed with a LabScan (Hunter Associates Laboratory, Reston, VA), which measures reflected rather than transmitted light. From these measurements both the optical densities and CIE **a** values were determined. All measurements with both instruments were made in triplicate and averaged. Lycopene contents of the homogenates were determined by extraction in hexane:ethanol (3:4) and spectrophotometric quantification (Barrett and Anthon, 2001).

RESULTS AND DISCUSSION

The ability of the UltraScan to determine lycopene levels was examined by preparing a series of homogenates from both fresh market and processing type tomatoes. Tomatoes were ground in a blender with various amounts of added water to produce homogenates with a range of lycopene contents, as determined by solvent extraction and spectrophotometric quantification. Following the procedure of Davis et al. (2003), the absorbance difference between 560 and 700 nm (A560-A700) was then measured in the UltraScan and compared with the lycopene levels (Fig. 1A). A linear relationship between the absorbance difference and the lycopene content was found, in agreement with the results of Davis et al. (2003). The slope of the regression line for homogenates prepared in a blender (0.0269) also agrees with the value reported by Davis et al. (0.030) for fresh tomato homogenates.

One difficulty encountered when preparing fresh tomato homogenates in a blender was the presence of air bubbles. Numerous air bubbles, which increase the light scattering and thus the measured absorbance of the sample, are present immediately after blending. To eliminate any effect these would have on the absorbance measurements, samples were allowed to sit for at least an hour between blending and absorbance measurements to allow the bubbles to dissipate. After this amount of time bubbles were no longer visually apparent and the measured absorbance values were stable. To avoid the introduction of bubbles we tried homogenizing the tomatoes with a mortar and pestle rather than a blender. Tomatoes homogenized in this way also showed a linear relationship between the absorbance difference and the lycopene content (Fig. 1B) but the slope of this line is only 0.0179 or 63% of that obtained when a blender was used (Fig. 1A). Apparently, for a given lycopene content in a tomato, the absorbance of a homogenate, measured in the UltraScan, depends on the method used to homogenize the tomato. Factors other than the lycopene content, such as the particle size and other physical properties of the solution, must also exert a substantial effect. The biggest apparent difference between these two sets of samples was the finer particle size of the blender samples.

A comparison of the complete absorbance spectra of a sample prepared in a mortar and pestle with that of one prepared in a blender gives some indication as to the origin of the discrepancy. Since blender samples give higher (A560-A700) values for a given amount of lycopene (Fig. 1), it was possible to select two samples, one prepared in a mortar and pestle and one prepared in a blender, that had very different lycopene contents (60.3 and 43.4 mg/L respectively), yet gave similar (A560-A700) values of approximately 1.0. The complete absorbance spectra of the two samples (Fig. 2) confirms that the absorbance difference between 560 nm and 700 nm is nearly the same (1.041 and 1.075) in each. The big difference between the two spectra is that the background absorbance in the blender-prepared sample is higher and rises much more sharply with decreasing wavelengths. This rising baseline indicates that, in this sample, more of the absorbance measured at 560 nm is actually from background absorbance not lycopene. This would give a larger (A560-A700) value and explain the similarity in this value for the two samples despite the difference in lycopene contents.

The same samples that were analyzed with the UltraScan were also examined with

a LabScan, which measures the amount of light reflected rather than absorbed. This is the commonly used method for measuring the color of tomato products for which results are reported as CIE **L, a, b** values or combinations of these parameters. A plot of the CIE **a** values versus lycopene content (Fig. 3A) shows that they are not linearly related to each other. A logarithmic regression line gives a reasonable fit to the data, which agrees with what others have reported (Arias et al., 2000).

A better correlation, although still not linear, was obtained by plotting optical densities rather than CIE **a** values. By analogy to the method used for UltraScan XE absorbances, the difference in optical densities between 560 and 700 nm was calculated to correct for optical density due to factors other than lycopene. A plot of this difference versus lycopene content (Fig. 3B) also gives a curve that can be fit with a logarithmic regression line. One advantage that the LabScan measurements appear to have over those obtained with an UltraScan is that the method used to homogenize the tomatoes has less of an effect on the measurement. Data from tomato homogenates prepared in both a blender and a mortar and pestle fall along the same regression line (Fig. 3) which was not the case when measurements were made in the UltraScan (Fig. 1).

In addition to looking at raw tomatoes, Davis et al. (2003) examined the use of an UltraScan to determine lycopene in thermally processed tomato products. They showed that the parameter (A560-A700) was approximately linear with the amount of lycopene in the sample, although the correlation was not as good as with raw tomato homogenates. Significantly, the slope of the regression line was different for thermally processed and raw tomatoes, with processed tomatoes giving only about 75% as much of an absorbance difference for the same lycopene content. To examine the effect of thermal treatment, we determined the lycopene contents of a number of tomato homogenates, prepared either from raw tomatoes or from tomatoes given a microwave hot-break. Two homogenates with identical lycopene contents, one raw and one hot-break, were then selected and the UltraScan absorbance and LabScan optical density spectra determined. The spectra of the two samples are quite different, in spite of the fact that the lycopene contents of the two are the same (Fig. 4). At all wavelengths, and especially below 600 nm, the absorbance of the hot-break juice was substantially lower than that of the raw juice (Fig. 4A). The parameter, proposed by Davis et al. (2003) as the measure of lycopene content, A560-A700, was only 59% as large for the hot-break sample versus the raw sample. Similarly, the optical density difference, OD560-OD700, was also only 56% as large in the hot-break versus the raw sample (Fig. 4B).

A possible explanation for this change in the absorbance spectrum due to heating may be that heat caused a significant amount of the *trans*-lycopene to isomerize to *cis*-lycopene, which has less absorbance. This does not appear to be the case, however. We measured the absorbance spectra of hexane extracts of the two samples and found them to be identical (data not shown). This indicates that no isomerization or other change in the lycopene resulted from the heating of the tomato juice. This agrees with the report of Nguyen and Schwartz (1998) who showed, by HPLC methods, that *trans*-lycopene does not isomerize to any significant extent during thermal processing of tomato juice. The reason why the same amount of *trans*-lycopene gives a very different absorbance spectra (and visibly different color) in raw and cooked juices is at present unclear but presumably reflects changes in the tomato matrix caused by heating.

CONCLUSIONS

If samples are homogenized under carefully controlled conditions and optimally diluted it is likely that lycopene contents can be estimated to within a reasonable error by absorbance or reflectance measurements. The successful application of these methods, however, requires that the tomatoes be thoroughly homogenized, accurately diluted, transferred to a glass cell, and read in an analytical instrument. This is almost as many steps as in simple solvent based spectrophotometric methods (e.g. Barrett and Anthon, 2001; Fish et al., 2002). Other than avoiding volatile organic solvents and their associated costs, it is hard to see much of an advantage to these methods. An inherent limitation in

their use is the need to construct a calibration curve, for which solvent extraction and spectrophotometric determination will still be needed. Furthermore, since properties of the tomato matrix can have a strong effect on the optical measurements, lycopene determined by these optical methods will always be less certain than those determined by solvent extraction and direct measurement.

ACKNOWLEDGEMENTS

The authors thank Gordon Leggett of Hunter Labs for the loan of the UltraScan XE used in this work. This work was supported by funds from the California League of Food Processors.

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Figures

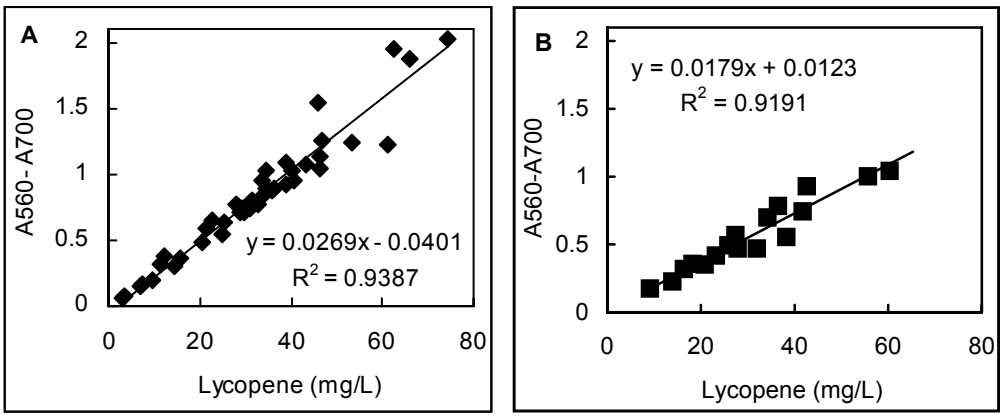

Fig. 1. Absorbance difference between 560 and 700 nm, measured in the UltraScan XE, versus lycopene content of the tomato homogenate. A. Tomatoes homogenized in a blender. B. Tomatoes homogenized in a mortar and pestle.

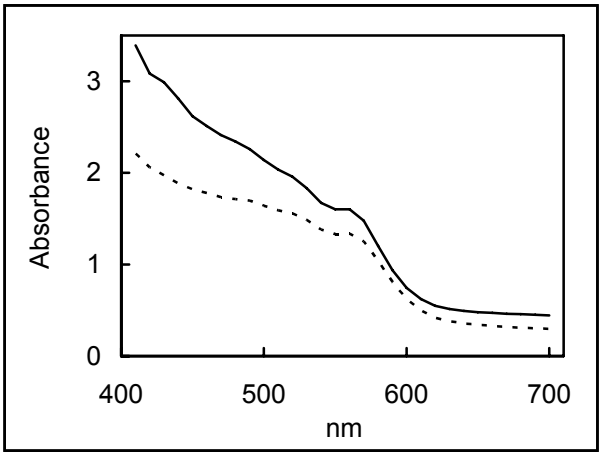

Fig. 2. UltraScan absorbance spectra of tomato homogenates prepared either in a blender (solid line) or mortar and pestle (dashed line). The lycopene content of the blender sample was 43 mg/L; the mortar and pestle sample contained 60 mg/L.

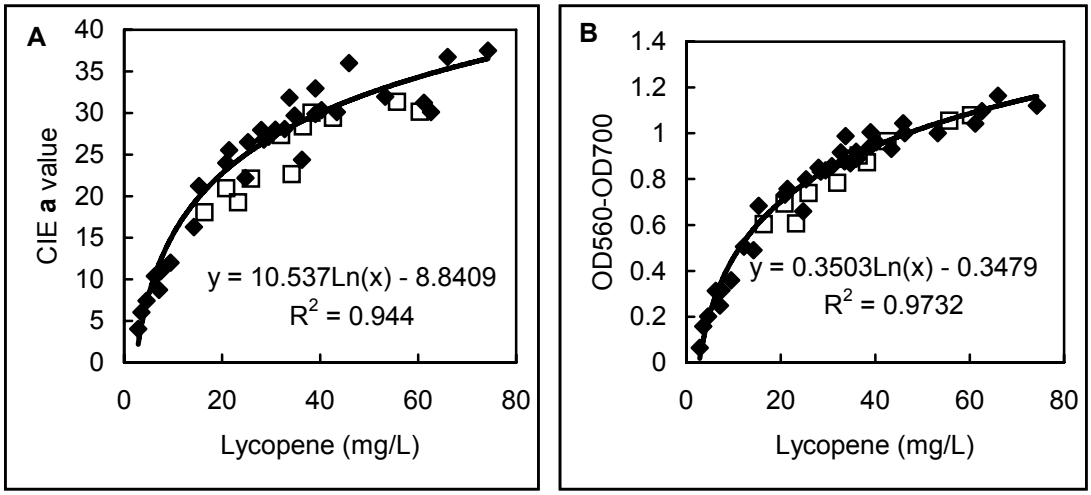

Fig. 3. Analysis of raw tomato homogenates with a LabScan. A. CIE **a** value versus lycopene content. B. Optical density difference versus lycopene content. Closed symbols indicate homogenates made with a blender; open symbols, homogenates made with a mortar and pestle.

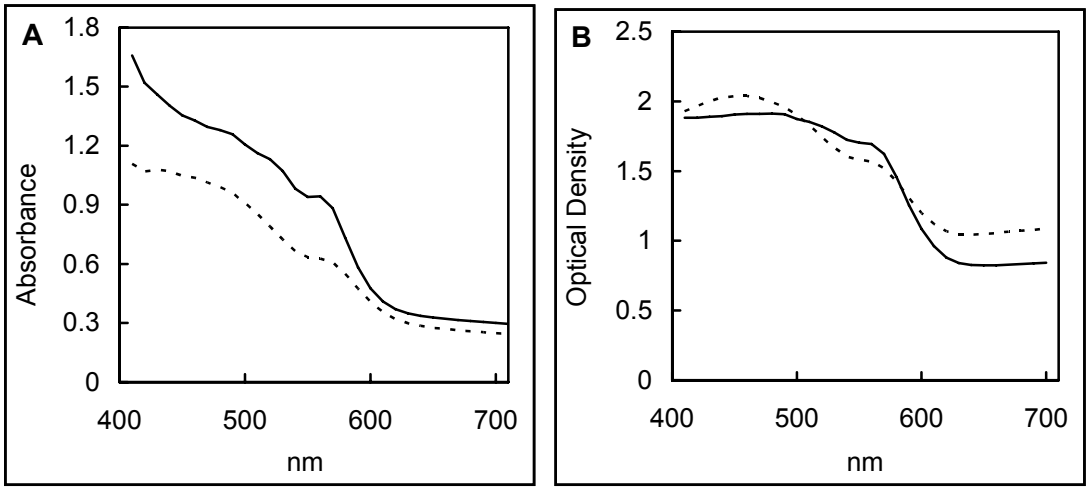

Fig. 4. Comparison of the spectra of raw (solid lines) and microwaved (dashed lines) tomato samples. Both samples contained 25 mg/L lycopene. A. Absorbances measured in the UltraScan. B. Optical density measured in the LabScan.