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A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products

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Abstract

Lycopene is a carotenoid that has antioxidant properties and imparts the red pigment in some fruits and vegetables. Tomato (*Lycopersicon esculentum* Mill.) is one of the predominant lycopene sources in a typical North American diet. Current methods to assay lycopene content in fruit are time consuming, expensive, and use hazardous organic solvents. Here, we report a method by which light absorbance measured with a scanning xenon flash colorimeter/spectrophotometer is used to quantify lycopene content in pureed translucent fruit samples. We evaluated 13 tomatoes (four different cultivars) and 38 tomato products. Our puree absorbance method (PAM) had linear correlation coefficients with lycopene content determined by hexane extraction/spectrophotometry of $R^2 = 0.97$ for fresh tomato, and 0.88 for tomato products. These linear correlations between methods show that this rapid method will likely work for quantitating lycopene content in purees of fresh tomatoes and some prepared foods. Since pureeing is the only processing required and no chemicals are needed, the method is rapid, inexpensive and requires no hazardous chemicals. Since lycopene has health benefits and food colorant potential, lycopene content in fruits is of interest to the food industry. This simple method for measuring lycopene content in a wide variety of foods and food products promises widespread use for lycopene quantitation.

Keywords: Lycopene; Tomato; Carotenoid; Lycopene assay; Lycopene quantitation; Lycopene detection method

1. Introduction

Lycopene, a fat soluble carotenoid, is a precursor of β-carotene (Sandmann, 1994) and has at least twice the antioxidant capacity of β-carotene (Di Mascio et al., 1989). Epidemiological studies have indicated positive health benefits in consumption of diets high in lycopene (Gerster, 1997). Since lycopene has value as a phytonutrient, many breeders want to maximize lycopene content in their breeding lines, and growers want to utilize production methods to increase lycopene content. Thus, simple and inexpensive assays to quantify lycopene are desirable prerequisites to developing

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produce with higher levels of this phytonutrient. Conventional spectrophotometric or HPLC assays to quantify lycopene from tissue utilize organic solvents to extract and solubilize this compound (Beerh and Siddappa, 1959; Adsule and Dan, 1979; Sadler et al., 1990). Although these methods are reliable, they are laborious, cumbersome, and require use and disposal of hazardous organic solvents. The need for specialized, expensive equipment and trained technicians for the current lycopene detection assays makes testing lycopene content impractical for some breeders, producers and researchers. Therefore, an easy, inexpensive, and reliable method to determine lycopene content over a broad spectrum of fruits and varieties that does not require hazardous chemicals is needed for the food industry. To this end, others have looked at the feasibility of using various tristimulus colorimeters to measure reflected visible color and to correlate reflectance values with lycopene content. D'Souza et al. (1992) showed that the lycopene concentration of tomato (*Lycopersicon esculentum* Mill.) can be estimated using a tristimulus colorimeter set to read in the CIE $L^*a^*b^*$ color scale using the equation $(a^*/b^*)^2$ (R^2 0.75). Arias et al. (2000) showed that a^*/b^* readings on tomato yielded a highly linear regression ($R^2 =$ 0.96) when compared with lycopene quantity. However, this study was conducted on only one variety and was based on a ripeness range of green to relatively low lycopene value red tomatoes. Thompson et al. (2000) showed, using multiple varieties, that CIE $L^*a^*b^*$ hue values from a tomato homogenate could be correlated (-0.85) to lycopene content better then surface reflectance readings. However, the correlation seemed to lose sensitivity in the red ripe stages.

In this report, we demonstrate the feasibility of quantitating lycopene content in the purees of tomato tissue from multiple varieties and many different tomato products with the use of a diode array xenon flash spectrophotometer. This method, reported for predicting watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) lycopene content (Davis et al., 2002), could allow fast and accurate quantitation of the lycopene content in tomato and tomato products.

2. Materials and methods

2.1. Sample preparation

All steps were performed in subdued lighting at room temperature. Tomatoes were purchased in a grocery store so variety names were not available. Of the tomatoes used there were cherry type, roma type and two different slicing type tomatoes. Individual tomatoes were sliced and all parts of the fruit were utilized. The fruit tissue was cut into approximately 1.4–2.6 cm cubes. Fresh tomato samples (25–500 g) were homogenized in a Waring blender until chunks were less then 4 mm^3. Canned products were used in toto and pureed without cutting. Fresh tomatoes were diluted 1:1 (W:V) in deionized water before blending and tomato products that had a thick consistency were also diluted with deionized water before pureeing (see Table 1) to make the samples translucent. All samples were pureed using a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, NY) with a 20 mm O.D. blade to produce a uniform slurry with particles smaller than 2×2 mm. The samples were not allowed to heat or froth.

2.2. Low volume hexane extraction method

The low volume hexane extraction method (LVHEM) was performed as in Fish et al. (2002). Approximately 0.6 g (determined to the nearest 0.01 g) duplicate samples were weighed from each puree into two 40 ml amber screw-top vials (Fisher, #03-391-8F) that contained 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% USP grade ethanol, and 10 ml of hexane. Purees were stirred on a magnetic stirring plate during sampling. Samples were extracted on an orbital shaker at 180 RPM for 15 min on ice. After shaking, 3 ml of deionized water were added to each vial and the samples were shaken for an additional 5 min on ice. The vials were then left at room temperature for 5 min to allow for phase separation. The absorbance of the upper, hexane layer was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was

Table 1
Tomato products diluted with deionized water and analyzed for lycopene content

Product name	Company	# of lots Tested	Concentration (%)[a]
Vegetable juice	A	2	33
Spicy hot vegetable juice	A	1	33
Tomato juice	A	2	33
Tomato soup	A	2	33
Tomato sauce	B	2	33
Tomato sauce	C	1	33
Tomato sauce	D	1	33
Tomato paste	C	1	25
Diced Tomatoes (peeled)	B	1	33
Tomatoes Stewed	C	2	50
Tomatoes Whole	C	1	50
Whole Peeled Tomatoes	E	1	50
Diced Tomatoes and Green Chilies	F	1	50
Chunky Tomatoes and Green Chilies	F	1	50
Fire Roasted Tomato Salsa	G	1	33
Taco sauce	H	2	33
Picante sauce	I	2	50
Tomato Ketchup	C	1	25
Tomato Ketchup	J	1	25
Tomato Ketchup	K	1	25
Pasta Sauce Traditional	L	2	33
Pasta Sauce Mushroom	L	1	33
Pasta Sauce Traditional	M	1	33

[a] Percent of tomato product (W:V) added to deionized water before samples were analyzed.

then estimated using the absorbance at 503 nm and the sample weight (Beerh and Siddappa, 1959; Fish et al., 2002).

2.3. Puree absorbance method

The Puree Absorbance Method (PAM) was performed as in Davis et al. (2002). The Hunter UltraScan XE was standardized as per company specifications each day the instrument was used. Purees were mixed well by gently shaking in a sealed plastic bottle and approximately 20 ml of each sample were immediately poured into a 1 cm, 20 ml SR101A cuvette (Spectrocell, Oreland, PA). Samples were scanned in the transmittance (TTRAN) mode under the following settings: large reflectance port (2.54 cm), Illuminant at D65, MI Illuminant Fcw, and observer 10°. The instrument was blanked on the empty cuvette. Triplicate readings were taken. For data analysis, absorbance at 700 nm was subtracted from absorbance at 560 nm. The LVHEM and the PAM were performed on the same day since storage alters the lycopene content in excised tissue (unpublished data).

2.4. Statistical analysis

Linear least square regression analysis, and mean and standard deviation determinations were performed using the statistical component of EXCEL software SR-1.

3. Results and discussion

Due to its design, the Hunter UltraScan XE subjects samples to light intensities that are orders of magnitude greater than those of analytical spectrophotometers with quartz halogen lamps and has sphere collectors that collect scattered as well as non-scattered light (Gorden Leggett, Hunter Associates Laboratory, Inc., personal communication). This has the potential to allow reliable

spectral measurements on translucent samples that scatter light. The spectra for the fresh tomato samples using this method, for which lycopene constitutes the predominant carotenoid content (Holden et al., 1999), exhibit apparent absorption maxima at 560, 520, and 490 nm. These maxima are decidedly red-shifted from the maxima at 505, 475, and 445 that are observed for lycopene in organic solvents such as hexane. They are, however, located at or near the same wavelengths observed for lycopene when it is extracted into aqueous solutions of dilute detergents (Fish, personal communication).

Based on the spectral results of lycopene in an aqueous phase (Fish, personal communication), we investigated the possibility of employing absorbance measurements at 560 nm of tomato and tomato product purees as a means to estimate lycopene content of the original sample. Samples include tissue from 13 tomatoes (four varieties) (Fig. 1), and 38 tomato products (Table 1, Fig. 2). The 51 tomato samples had a lycopene concentration range on a fresh weight basis from 6.6 to 490 mg kg^{-1} in the original tissue. The lycopene concentration in the diluted samples that were run in the PAM and LVHEM ranged from 3.3 to 123 mg kg^{-1} FW. The absorbance of each sample was measured for each puree at 560 nm and adjusted for scatter by subtraction of the absorbance at 700 nm. The adjusted measurements were plotted against the sample's lycopene content as measured by hexane extraction (Figs. 1 and 2). The scatter-adjusted absorbances at 560 nm appear to obey Beer's law with respect to lycopene content of the puree. The absorbance reading is linearly correlated with lycopene content, the linear least squared fit to the plotted data had an R^2 value of 0.96 for fresh tomato samples and an R^2 of 0.88 for tomato products. The linear least squares fit to the fresh tomato samples' data yielded the equation: $y = 33.402x + 0.1988$ (Fig. 1). This can be used as a predictive equation for lycopene content in tomato tissue, by measuring the scatter-adjusted absorbance (560–700 nm) of the tomato purees and inserting each value into the linear equation. This was shown to work for watermelon by Davis et al. (2002).

The PAM is a rapid, accurate method to estimate the lycopene content in fresh tomatoes and can likely be utilized for quantifying lycopene content in processed foods once parameters are optimized for each product. The outlier in Fig. 2 is tomato paste and is likely not diluted enough. Removing this point raises the R^2 to 0.9, which shows that standardized procedures will need to be developed for each tomato product.

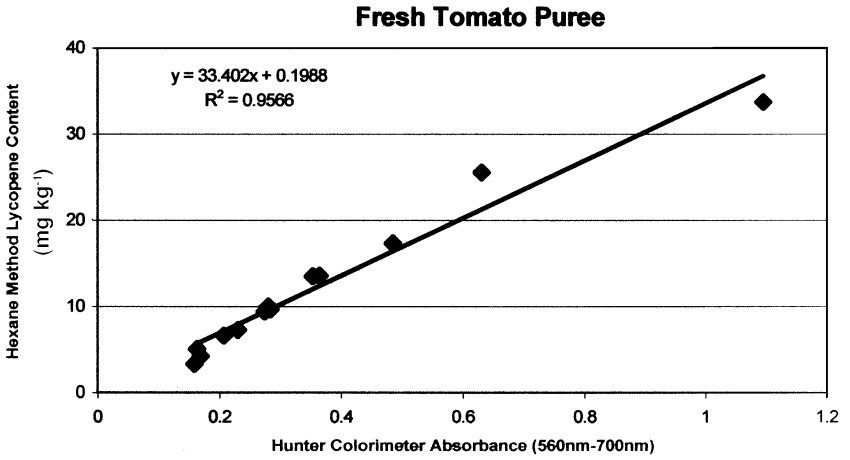

Fig. 1. Correlation of PAM with the LVHEM on thirteen tomato fruit purees. The absorbance recorded by the Hunter Lab UltraScan XE on diluted samples is plotted vs. the lycopene content of the same diluted sample determined by the LVHEM. The absorbance at 560 nm is adjusted for scatter by subtracting the absorbance at 700 nm. The R^2 value and the linear least squares fit equation are given in the figure.

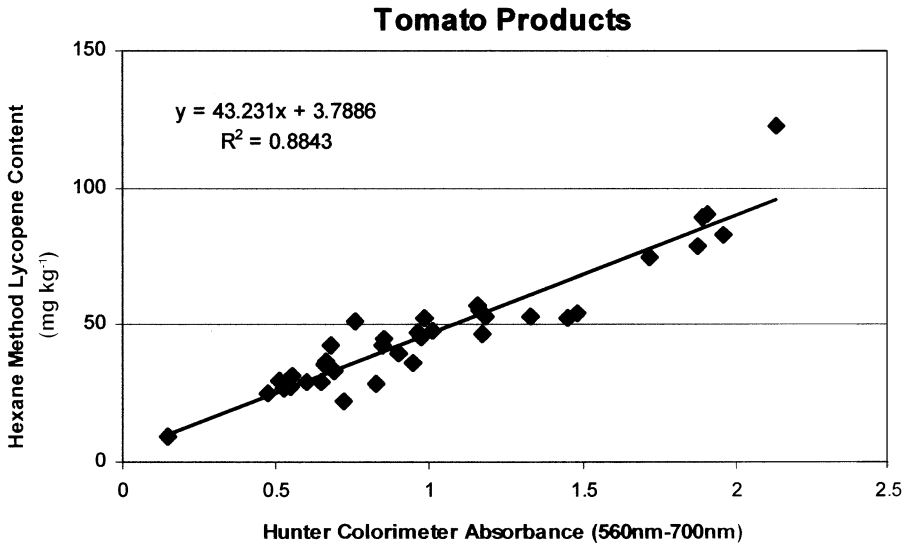

Fig. 2. Correlation of PAM with the LVHEM on 38 tomato product (Table 1) purees. The absorbance recorded by the Hunter Lab UltraScan XE on diluted samples is plotted vs. the lycopene content of the same diluted pureed tomato products determined by the LVHEM. The absorbance at 560 nm is adjusted for scatter by subtracting the absorbance at 700 nm. The R^2 value and the linear least squares fit equation are given in the figure.

In this research, we diluted the samples to make a translucent liquid that could be read through a 10 mm cuvette. Concurrent experiments showed that dilution of tomato samples did not change the predicted lycopene content of the original product using the hexane extraction method. However, a 1 mm cuvette might work for pourable tomato samples without having to dilute the sample. Lycopene is the predominant pigmented compound in red tomatoes, but other carotenoids such as β-carotene, α-carotene, and lutein constitute about 20% of the total carotenoids in fresh red tomato tissue (Holden et al., 1999). Fish et al. (2002) suggest that for the levels and the 560 nm extinction coefficients of these minor carotenoids, ignoring their contribution in this lycopene assay will result in an over estimation of lycopene content of less than four percent. However, experiments with orange and yellow tomatoes would need to be performed to verify this.

The precision of the PAM appears to be comparable to that of the conventional hexane assay. For samples assayed by the PAM, the average standard error per triplicate readings (560–700 nm) was ±0.01% for the 13 fresh tomato samples and ±0.01% for the 38 tomato product samples. The average standard error per duplicate sample was ±0.02 for the 13 fresh tomato purees and the 38 tomato product samples using the low volume hexane method. As a practical estimate of the average deviation from the least squares fit that may be anticipated for absorbance measurements of purees, the standard error of absolute deviation of each point from the straight line was determined and the mean standard error was 14.4% for the fresh tomato purees and 11.5% for the tomato product purees.

As with any quantitative assay procedure, to achieve the desired level of reliability, several steps in the assay require attention. The three critical steps in the puree absorbance procedure are 1) maintenance of subdued light while working with the sample since light degrades lycopene, 2) thorough homogenization of the tissue, and 3) thorough mixing of the puree while pouring and reading the samples. This latter step is important since the puree from some samples rapidly (~ 30 s) separates into layers of juice and pulp, and this separation introduces considerable error in the absorbance reading. Until such time as it can be

determined if all instruments of the type employed herein give identical responses to a given level of lycopene, a response curve like that in Fig. 1 will have to be generated for each instrument using an assay method such as hexane extraction/absorbance.

4. Conclusion

In the experiments presented, we demonstrate that the PAM gave a linear relationship to lycopene content and is independent of lycopene concentration, tomato variety, or tomato product. This method offers the opportunity to change lycopene quantitation from a cumbersome and slow method that requires hazardous reagents to one that is streamlined, requires no added reagents, and reduces sample processing time by at least half over the LVHEM (Fish et al., 2002). Savings on purchase and disposal of hazardous organic solvents alone will save over US $1 per sample. Such simplicity, efficiency, and safety should expedite germplasm screening and evaluation of environmental effects on maximal lycopene production for growers, breeders, and research scientists.

5. Disclaimer

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