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# Refinements of the attending equations for several spectral methods that provide improved quantification of β-carotene and/or lycopene in selected foods☆

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ABSTRACT

Developing and maintaining maximal levels of carotenoids in fruits and vegetables that contain them is a concern of the produce industry. Toward this end, reliable methods for quantifying lycopene and β-carotene, two of the major health-enhancing carotenoids, are necessary. The goal of this research was to rigorously refine the attending equations for existing spectral methodologies in order to more accurately quantify lycopene and β-carotene in selected fruits and vegetables. Equations taking into account two absorbing species with overlapping spectra were derived for three spectral methodologies. Carotenoid values determined by absorbance measurements in hexane with its attending equations provided one-to-one correspondence with values determined by reversed phase high performance liquid chromatography for lycopene and/or β-carotene in a broad spectrum of fruits and vegetables. Estimates for lycopene in hexane extracts averaged ±7.8% deviation from those by HPLC while estimates for β-carotene averaged ±5.0% deviation. Simple empirical relationships developed from correlating large numbers of xenon flash spectrophotometry data appeared to be nearly as reliable as the derived equation to treat the experimental data. Estimates for lycopene in watermelon flesh purees by the derived equation deviated ±6.8% from those estimated by HPLC while deviations by a currently employed empirical equation were ±10.8%. Absorbance measurements of chromoplasts suspended in aqueous sodium dodecyl sulfate generally required correction for light scattering by the chromoplasts, and equations derived for two absorbing species provided for quantification of lycopene and β-carotene by this method. Estimates for lycopene in watermelon flesh by the derived equations averaged ±9.3% deviation from those by HPLC while estimates for β-carotene in cantaloupe averaged ±12.0% deviation from HPLC values.

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## 1. Introduction

Many fruits and vegetables contain nutritionally beneficial quantities of the carotenoids, lycopene and β-carotene. Watermelon (Perkins-Veazie et al., 2001), red navel oranges (Lee, 2001), and tomato (Baysal et al., 2000) possess substantial quantities of each; red grapefruit and autumn olive berries (Fordham et al., 2001) possess mostly lycopene; and cantaloupe, mango, and sweet potato possess a preponderance of β-carotene (USDA National Nutrient Database, 2009). Plant breeders are interested in developing cultivars with increased carotenoid levels while produce handlers and food processors are concerned with keeping carotenoid loss at a minimum during postharvest produce handling and food processing (Sharma and LeMaguer, 1996). These efforts represent a desire to meet the expectations of ever-increasing numbers of health-conscious consumers. Methodologies have been developed in the past 15 years that provide simpler, safer, less expensive, and more environmentally friendly procedures to quantify carotenoid levels in foods.

High performance liquid chromatography (HPLC) provides both separation and quantification of individual carotenoids (Craft, 2001), but it requires considerable technical expertise, expensive instrumentation, and the use of hazardous solvents. Although it is the research method of choice, HPLC is too slow and expensive for routine screening of large numbers of samples.

Conventional solvent extraction coupled with spectrophotometry (Beerh and Siddappa, 1959; Adule and Dan, 1979; Sadler et al., 1990; Fish et al., 2002) has long been a relatively fast and reliable method. However, the methodology requires the use of flammable,

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biologically hazardous solvents that pose personnel safety and environmental waste issues.

A recent methodology, xenon flash spectrophotometry, has been shown to measure lycopene directly in pureed fruit (Davis et al., 2003a,b) and shows promise for measuring β-carotene in cantaloupe (Davis et al., 2008). The method is rapid, simple, requires little sample preparation, and uses no hazardous chemicals. It is somewhat disadvantaged by the cost of the equipment, and each instrument must be calibrated with tissue carotenoid values determined by HPLC or solvent extraction/spectroscopy. Fiber optic visible reflectance spectroscopy (Choudhary et al., 2009) offers a lower cost instrument alternative to xenon flash spectrometry. By taking the logarithm of the reciprocal of the puree reflectance value, an apparent absorbance value is obtained that was shown to be linearly related to the level of lycopene in watermelon or tomato purees (Choudhary et al., 2009). As with xenon flash spectrometry, this methodology measures lycopene while it is still inside the chromoplasts released in a fruit puree and must be calibrated with tissue values determined by another method.

Another recently reported methodology relies on the extraction of carotenoid-containing chromoplasts into an aqueous solution of the anionic detergent, sodium dodecyl sulfate (SDS), followed by spectral quantification of the carotenoids inside the chromoplasts (Fish and Davis, 2006; Fish, 2006, 2007). Though the method is simple, safe, environmentally friendly, and can be performed with the use of inexpensive equipment, light scattering by the chromoplasts can confound the accuracy of the measurement.

With the exception of HPLC, all of the current spectral methods to quantify carotenoids are affected by the presence of appreciable quantities of multiple carotenoids whose spectra partially overlap with that of the carotenoid to be quantified. For example, increased β-carotene levels in watermelon or tomato diminish the accuracy of lycopene estimates. In an effort to improve the reliability of these various spectral methods for β-carotene and lycopene quantification, this study had two objectives. The first objective was to refine the attending equations for each methodology in order to take into account the potential presence of appreciable quantities of both carotenoids. The second objective was to critically compare the reliability of results obtained with these new methods of data treatment with results from data treatment methods currently practiced.

## 2. Materials and methods

### 2.1. Plant material and sampling

Watermelon and cantaloupe used for the study were grown on research plots at the Wes Watkins Agricultural Research Laboratory, Lane, OK, or were purchased from a local supermarket. Tomatoes, mangos, pink grapefruit, and sweet potatoes were purchased from the supermarket.

In most instances, tissues were processed and analyzed without freezing. Forty gram samples were removed from the heart of ripe watermelon or from the flesh tissue of ripe cantaloupe and immediately ground to a homogeneous puree with a Polytron homogenizer (Brinkman Polytron Homogenizer, Westbury, NY) for 1 min at a speed setting of 4. Fresh tomatoes were finely ground to a puree in an equal weight of deionized water with a Polytron homogenizer at a speed setting of 8 for 2 min before sampling and assay. The peel and seeds were removed from fresh grapefruit tissue and fresh mango tissue before the flesh was finely ground with the Polytron homogenizer for 1 min at a speed setting of 4. Sweet potato was peeled, 100 g of tissue were cut into 1/2 cm cubes, and these were ground in a food grinder (Weston Products, Strongville, OH). To the ground sweet potato tissue was added twice its weight of deionized water, and the mixture was finely ground with the Polytron homogenizer for 3 min at a speed setting of 10 before sampling for analysis. In some instances, watermelon or cantaloupe purees were frozen and stored at −80 °C to be re-analyzed after a brief storage at this temperature. Tissue purees were kept in reduced room light after preparation and until assayed. Purees were stirred on a magnetic stirring plate during replicate sampling. Before HPLC analysis, mango samples were first hydrolyzed by incubation with 5% KOH in 50% methanol–50% hexane with stirring at 25 °C for 16 h (Craft, 2001). The organic layer was washed twice with equal volumes of deionized water before sampling and/or assay.

### 2.2. Combinatorial experiment

As a critical test of the methodologies and their attending equations, a combinatorial experiment was performed wherein tissues from fresh watermelon (var. Obsession: [Lyc] = 92.8 mg/kg and [β-Car] = 3.1 mg/kg) and fresh cantaloupe (var. Magnum 45: [β-Car] = 23.1 mg/kg) were combined in various weight ratios, ground to homogeneity, and assayed by the different methods described in the following.

### 2.3. HPLC analysis of carotenoids

Quantification of carotenoids by HPLC was performed as generally outlined by Craft (2001). Runs were conducted on an Agilent Technologies Model 1100 system fitted with a diode array detector. Separation was carried out on a 3 μm 250 mm × 4.6 mm C30 column (Waters Corp., Milford, MA) at 25 °C. Carotenoids were eluted with the ternary gradient program described by Craft (2001). *Trans*-β-carotene standard was prepared from cantaloupe chromoplasts (Fish, unpublished) and *trans*-lycopene standard was prepared from watermelon chromoplasts (Fish, 2006). Standards were prepared by extraction into hexane, the concentration determined spectrophotometrically using published absorptivities and Eqs. (1) and (2) (Zechmeister and Polgar, 1943; Zechmeister et al., 1943), and a purity correction applied from the HPLC chromatograms as outlined by Craft (2001). The purity correction for cantaloupe chromoplast *trans*-β-carotene was 97.2%, and the purity correction for watermelon *trans*-lycopene was 91.8%. *Cis*-isomers of lycopene were quantified using the same integration constant as for all-*trans* lycopene. Because this methodology extracts the carotenoids into an organic solvent, it proved to work equally well on all samples tested.

### 2.4. Reduced hexane volume lycopene and β-carotene assays

This procedure followed that outlined by Fish et al. (2002). Each sample was assayed in triplicate. A given volume of sample was pipetted from the stirred puree into a tared vial, and the sample mass was determined to the nearest 0.001 g. Depending upon the carotenoid content, the amount of sample used in the reduced volume assay ranged from 0.4 to 0.6 g. At this range of sample mass, it was imperative to record the mass of sample added to the assay vial to the nearest 0.001 g in order to maintain an acceptable level of precision. For the assays, 0.1 M NaCl solution was used instead of distilled water to improve the rate of phase separation and diminished the occurrence of emulsions at the solvent–water interface. If necessary, an additional 5 mL of ethanol added to the extraction system improved phase separation for samples highly prone to emulsification. This occasionally occurred with over-ripe fruit. The absorbance of the hexane (upper) layer was measured in a 1 cm path length quartz cuvette at 503 nm and 450 nm versus a blank of hexane solvent.