A Rapid Spectrophotometric Method to Determine β-Carotene Content in *Cucumis melo* germplasm

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Abstract: β-carotene is a carotenoid that has antioxidant properties, is a precursor of Vitamin A, and imparts the orange color in some fruits and vegetables. This compound is the major carotenoid in cantaloupe. Because of its health benefits, the β-carotene content in fruits is of interest to the food industry and to melon breeders. Current methods to assay β-carotene content in fruit are time consuming, expensive, and use hazardous organic solvents. In this report, preliminary data is shown for a method to quantify β-carotene content of cantaloupe puree using light absorbance measured with a xenon flash colorimeter/spectrophotometer. Absorbance of twenty seven cantaloupe purees from one variety demonstrated a linear correlation coefficient (R^2=0.7) with β-carotene content determined by hexane extraction/spectrophotometry. This linear correlation shows that this method may be suitable for quantifying β-carotene content in purees of fresh cantaloupe. Since pureeing the sample is the only processing required and no chemicals are needed, the method is rapid, inexpensive and produces no hazardous waste.

Materials and Methods: *Sample Preparation.* All steps were performed under subdued lighting at room temperature. Cantaloupe flesh tissue was cut into approximately 2 to 4 cm cubes. Samples (25 to 500 g) were homogenized in a Waring blender until particle sizes were less than 4 x 4 mm. All samples were then pureed using a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, New York) with a 20 mm O.D. blade to produce a uniform slurry with particles smaller than 2 x 2 mm. The samples were not allowed to heat or froth. A water soluble form of β-carotene was diluted in water to use as a control (BASF The Chemical Company, Ludwigshafen, Germany).

Low Volume Hexane Extraction Method (LVH): The low volume hexane extraction method was performed as in Fish et al. (2002). Approximately 0.6 g (determined to the nearest 0.001 g) duplicate samples were weighed from each puree into 2 forty ml amber screw-top vials (Fisher, #03-391-8F) that contained 5 ml of 0.05% (w/v) BHT in acetone, 5 ml of 95% 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 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 479 nm blanked with hexane. The β-carotene content was then estimated using absorbance at 479 nm and factoring in the sample weight (Zechmeister and Polgar 1943; Beerh and Siddappa 1959; Fish et al. 2002).

Puree Absorbance Method: The puree absorbance method was modified from a lycopene detection method in watermelon and tomato (Davis et al. 2003a, b). Briefly, 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 the 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: the large reflectance port (1.00"), Illuminant at D65, MI Illuminant Fcw, and observer 10°. The instrument was blanked on the empty cuvette. Triplicate readings were taken. Absorbance at 750 nm was subtracted from absorbance at 520 nm for analysis.

Results and Discussion: *Absorbance of β-carotene standard in water.* A serial dilution in water of a BASF β-carotene standard was performed. An aliquot was read using the LVH method to check for accurate preparation for each dilution. Additionally, each dilution was read on the UltraScan XE and the absorbance was compared to the percent of the standard starting solution and the measured β-carotene concentration using the LVH method. The UltraScan XE readings to the LVH estimated β-carotene concentrations were compared (Figure 1). This figure demonstrates that the BASF standard follows the Beer-Lambert law when diluted in water and when read on the UltraScan XE up to an absorbance of three, which is the ceiling for this instrument. This data also demonstrates that the UltraScan XE provides more consistent readings than the LVH method. This finding indicates that an aqueous fruit puree should also obey the Beer-Lambert law.

Absorbance behavior of puree as related to β-carotene content: Based on spectral results, we investigated the

possibility of employing absorbance measurements at 430 nm, 490 nm, and 520 nm for cantaloupe puree as a means to estimate the β-carotene content. Samples included tissue from 27 cantaloupe fruit (1 variety). The absorbance reading of the puree at 520 nm gave a higher correlation with the hexane extraction method.

The absorbance at 520 nm measured for each puree as adjusted for scatter by subtraction of the absorbance at 750 nm was plotted against its β-carotene content as measured by hexane extraction (Figure 2). The scatter adjusted absorbances at 520 nm of the purees appear to obey Beer's law with respect to β-carotene content of the puree. The absorbance reading is linearly correlated with β-carotene content, and the linear least squares fit to the data yields the equation: $y = 23.694x + 5.7785$.

Freeze-thawed samples can not be compared with fresh samples. The freeze-thawed samples exhibit a different conversion equation than fresh samples (Data not shown.). For each level of β-carotene, fresh samples read with a higher absorbance than frozen samples. This is likely due to protein and cell wall breakdown in the frozen tissue

Conclusions: In the search for a rapid and reliable way to quantitate β-carotene levels in cantaloupe tissue for screening large numbers of germplasm samples, we are developing a method that utilizes an instrument that can measure actual light absorbance of compounds in a slurried, aqueous medium. The method is simple, uses no hazardous chemicals, and is faster and less expensive than currently used methods. More cantaloupe varieties are being evaluated to determine the accuracy of this method.

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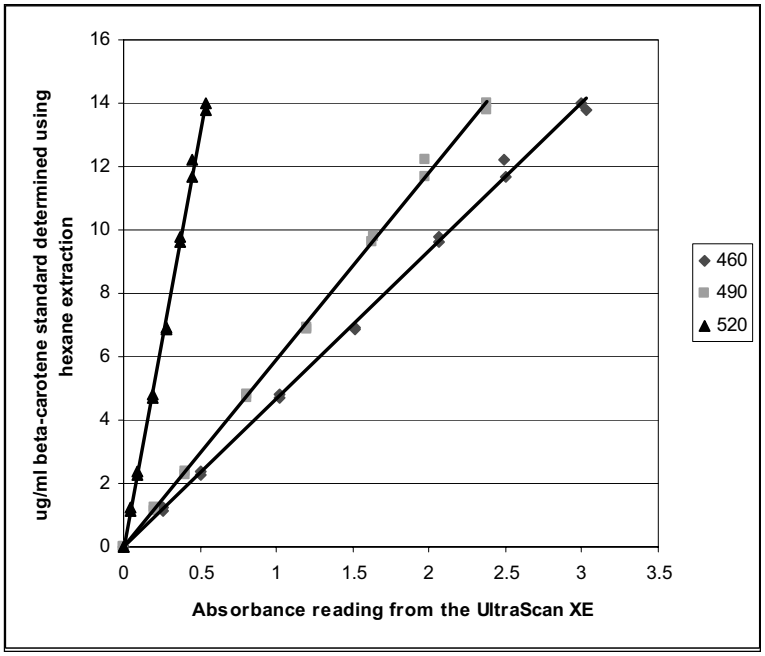

Figure 1: Demonstration of a β-carotene standard obeying Beer-Lambert law when diluted in water and analyzed using the UltraScan XE. Wavelengths 460 and 490 nm were chosen since they provided the highest readings of the scanned standard and the cantaloupe purees. Wavelength 520 nm was chosen because cantaloupe shows a peak at this wavelength. R^2 value for each linear least squared best fit line were all 0.99.

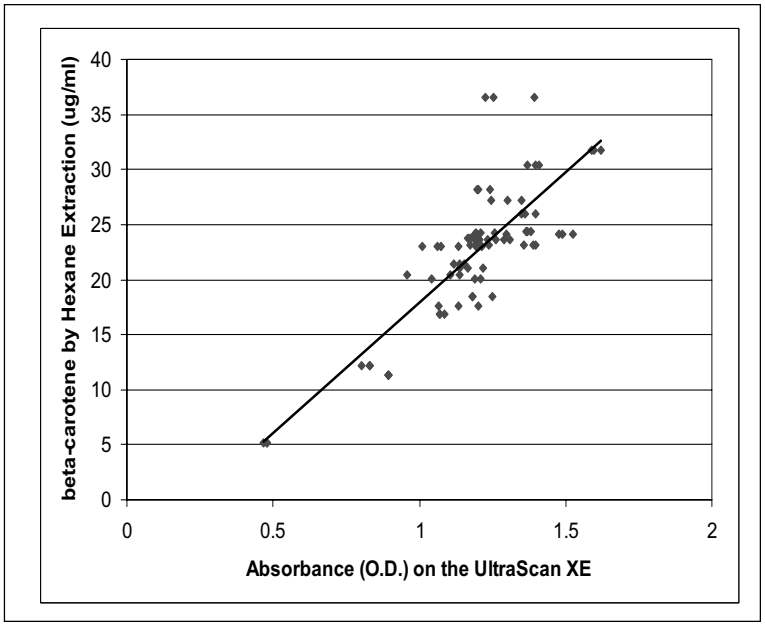

Figure 2: Results of absorbance of 27 cantaloupe purees for β-carotene using the Hunter Lab UltraScan XE. Absorbance is plotted *versus* the β-carotene content of the cantaloupe determined by the low volume hexane method. Absorbance at 520 nm was adjusted for scatter by subtracting the absorbance at 750 nm. The absorbance reading was linearly correlated with β-carotene content, and the linear least squares fit to the data yielded the equation: y = 23.694x + 5.7785. The R^2 value for this linear correlation was 0.7.