

# Green-Fleshed Watermelon Contains Chlorophyll

**Angela R. Davis, Penelope Perkins-Veazie**

USDA, ARS, South Central Agriculture Research Laboratory, P.O. Box 159, Lane, OK 74555 U.S.A.

**Stephen R. King**

Vegetable & Fruit Improvement Center, Department of Horticultural Sciences, Texas A & M University, College Station, TX 77843-2133 U.S.A.

**Amnon Levi**

USDA, ARS, United States Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC, 29414-0000 U.S.A.

Many popular and technical reports on watermelon flesh colors ignore green, an uncommon color. The earliest report of this color mutant that we were able to find dates back more than one hundred years. In this report, inheritance of pink-fleshed vs. green-fleshed genes in watermelon was explored and resulted in fruit intermediate in character, the flesh having a yellowish cast tinged with pink (Card and Adams, 1901). Years later, Whitaker and Davis (1962) listed greenish-white as one of the flesh colors found in watermelon, as well as white, yellow, and red. More recently, Robinson and Decker-Walters (1997, page 84) wrote, "The bland to sweet-tasting flesh is usually red, but may be green, orange, yellow or white in some cultivars or landraces." They also mention, "Citron has white or pale green flesh which is bland or bitter" (p.97). Additionally, green is one of the eight flesh colors and color combinations listed in the Germplasm Resources Information Network for *Citrullus* spp. (USDA, ARS, GRIN). In addition to the rare reports of green color, the compound giving this greenish cast has not been mentioned.

Since some cucurbits have chloroplasts and thus chlorophyll inside their fruit, we surmised that the green may be chlorophyll. We analyzed fifteen *Citrullus* spp. that demonstrated white to greenish-white flesh to determine if chlorophyll is present.

Quantification of chlorophyll was performed on 14 greenish-white watermelon flesh samples. The sample came from citron type PI lines 271769 (8 fruit), 271773 (1 fruit), and 299378 (2 fruit); *lanatus* type PI line 494531 (1 fruit); and volunteers that resembled citron types (3 fruit). Three of the greenish-white flesh samples had no detectable amounts of total chlorophyll and were probably below the detectable limits using our spectrophotometric analysis. The remainder of the samples, including the *lanatus* type, contained very low levels, from 1 to 6 µg/g total chlorophyll. This is roughly 7% of

the amount of chlorophyll found in fresh broccoli florets.

Three of the greenish-white samples above (one 271769 and two 271773) were separated using high performance liquid chromatography (HPLC); two white samples were also run using HPLC ('Cream of Saskatchewan' and PI 314655). Comparison of the sample peaks to chlorophyll standards verified chlorophyll peaks in three greenish-white samples. The white-fleshed watermelon samples gave no detectable chlorophyll peaks. These results suggest that chlorophyll is imparting the green tint to greenish-white-fleshed *Citrullus* spp.

We know that carotenoids in watermelon are packaged in chromoplasts, similar to red tomato (Fish, 2006; Harris and Spurr, 1969), but we found no reports that watermelon flesh contains chloroplasts. Our finding suggests that *Citrullus* spp. containing chlorophyll also contain chloroplasts.

If there are indeed chloroplasts in watermelon flesh, this generates more questions. Is chlorophyll present in all watermelon or only the ones with a greenish tint? How much chlorophyll does watermelon have the potential to make? Is chlorophyll or the other components of chloroplasts, such as violaxanthin, affecting the complexity of color in red, yellow, and orange watermelon? Are the chloroplasts functional? Does light penetrate the rind and does the presence of chlorophyll in the flesh help the fruit synthesize energy and sugars?

**Materials and Methods:** *Plant material.* Ripe watermelons of white to greenish-white were grown at Lane, OK, and in College Station, TX from 2005-2007. Five Plant Introduction lines (PI) (271773, 271769, 299378, 314655, and 494531), one open pollinated variety ('Cream of Saskatchewan'), and three volunteers of unknown origin were evaluated for chlorophyll. Fruits were cut the day of harvest and flesh tissue was extracted from the heart.

*Sample Preparation:* Immediately after collecting heart tissue the samples were frozen and stored at -80°C until processed for spectrophotometric and HPLC analyses. Tissue (~30 g) was homogenized 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 3 mm<sup>3</sup>. For HPLC analyses, samples were concentrated by centrifugation and then extracted. Samples analyzed using a spectrophotometer were not concentrated before extraction. Samples were extracted using a modified acetone extraction method (Lichtenthaler, 2001).

*HPLC and spectrophotometric analysis:* A subset (three) of the samples tested above and two white-fleshed samples were concentrated and then extracted as above and analyzed following HPLC methods previously described (Craft, 2001). Samples were filtered using 0.45 µm PTFE syringe filters (Daigger, Vernon Hills, IL) into 2-ml amber crimp-top vials (Daigger, Vernon Hills, IL), then loaded onto an Agilent model 1100 high performance liquid chromatography system equipped with autosampler, photodiode array detector, and integration software (Agilent model 1100, Wilmington, DE). A C30 YMC carotenoid column (4.6 x 250 mm) and YMC carotenoid guard column S-3 (4.0 x 20 mm) (Waters, Milford, MA) was used. A gradient method with three solvent mixtures was used for separation. Solvent mixtures of (A) 90% methanol, 10% ddi water containing 0.5% triethylamine and 150 mM ammonium acetate, (B) 99.5% 2-propanol, 0.5% triethylamine, and (C) 99.95% tetrahydrofuran, 0.05% triethylamine were applied as follows: initial conditions 90% solvent A plus 10% solvent B; 24 minute gradient switched to 54% solvent A, 35% solvent B and 11% solvent C; final gradient conditions were 11 minute gradient of 30% solvent A, 35% solvent B, 35% solvent C, held for 8 minutes. The mobile phases were returned to initial conditions for 15 min. Injection volumes of 100 µl were used for samples and standards. Chlorophyll A and B standards were obtained from Sigma (St. Louis, MO) and were used for peak verification.

Chlorophyll A and B were quantified using the spectrophotometric assay in Wrolstad et. al. (2004).

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Figure 1. Green-fleshed watermelon on the left and white-fleshed watermelon on the right. The white-fleshed watermelon also demonstrates the Egusi seed characteristic.



Figure 2. A range of watermelon fruit colors starting in the upper left-hand corner with pure white flesh, and ending in the lower right-hand corner with intense green. Intermediate amounts of green color as shown in the remaining three fruit.