

# Lack of Correspondence between Experimentally Determined Values of Vitamin E in Carrot (*Daucus carota* L.) and Those Reported in the USDA National Nutrient Database

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**Abstract.** Vitamin E compounds, known collectively as tocochromanols, are essential nutrients found in plant tissues. These compounds are present in fruits and vegetables at lower levels than in nuts and oils. However, since fruits and vegetables are frequently consumed in the diet, they represent important contributions to vitamin E intake. Knowledge of nutrient levels in fruits and vegetables is important in making dietary recommendations and in planning menus. The U.S. Department of Agriculture (USDA) National Nutrient Database (NNB) reports levels of vitamin E in carrot of  $6.6 \mu\text{g}\cdot\text{g}^{-1}$  on a fresh weight basis and similar levels of vitamin E for food products containing carrots, such as baby food and carrot juice. We collected data on four tocochromanol compounds using reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection on both fresh carrots and on commercially available food products containing carrots. Our data revealed that levels of vitamin E in fresh carrot and food products containing carrot were in the range of  $0.007\text{--}0.12 \mu\text{g}\cdot\text{g}^{-1}$  converted to a fresh weight basis. These levels were consistent with several published research studies for carrot, lower than several other published studies, and significantly lower than values reported in the NNB. Data from our studies show actual vitamin E values for this vegetable may be significantly lower than levels published in the NNB, but large discrepancies exist in the published reports for measurement of tocochromanol levels for this vegetable. Dietary guidance based on vitamin E values for carrot reported in the NNB could lead to inaccurate nutrient recommendations and should be clarified and standardized.

Vitamin E is a group of eight lipid-soluble antioxidants ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and tocotrienols, collectively known as tocochromanols) that are synthesized in the plastids of plants, algae, and some cyanobacteria (Mène-Saffrané and DellaPenna, 2010). Tocochromanols result from the condensation of homogentisate and phytyl pyrophosphate derived from the shikimate pathway and the nonmevalonate 1-deoxy-D-xylulose-5-phosphate pathway, respectively (DellaPenna

and Pogson, 2006). Vitamin E is an essential nutrient in the human diet, critical for membrane integrity and stability, and has a variety of health promoting properties (Hunter and Cahoon, 2007). Seeds, nuts, and oils are recognized as good sources of vitamin E and some orange and red vegetables and leafy greens have been noted to contain vitamin E. Murphy et al. (1990) found that vegetables were the second largest contributor, after fats and oils, to vitamin E intake in the adult U.S. diet, representing 15.1% of the total vitamin E contribution. Even though nuts and seeds are rich sources of vitamin E, they are consumed by fewer people and in smaller amounts than other vitamin E sources, and contribute  $\approx 4$ -fold less to total vitamin E intake (Murphy et al., 1990).

Carrots (*Daucus carota* L. var. *sativa*), well known for high concentrations of provitamin

A carotenoids, also contain vitamin E. Koch and Goldman (2005) and Luby et al. (2014) have reported several tocochromanols in carrot, including  $\alpha$ -,  $\beta$ - and  $\gamma$ -tocopherol and  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocotrienol. Burns et al. (2003) and Koch and Goldman (2005) used reverse-phase HPLC equipped with an ultraviolet detector to quantify vitamin E levels in carrot roots. These studies reported values of  $0.03\text{--}0.11 \mu\text{g}\cdot\text{g}^{-1}$  of total  $\alpha$ - and  $\beta$ -tocopherol on a fresh weight basis. Luby et al. (2014) used more sensitive and specific fluorescence detection during HPLC analysis, which was capable of measuring additional tocotrienols and tocopherols. Luby et al. (2014) measured an average of  $0.12 \mu\text{g}\cdot\text{g}^{-1}$  converted to a fresh weight basis of total tocochromanols in hybrid carrot roots. Ombodi et al. (2014), Metzger and Barnes (2009), and Nicolle et al. (2004) measured tocochromanols in carrot using various techniques. These three studies revealed levels of tocochromanols in carrot that were significantly higher than those reported by Burns et al. (2003), Koch and Goldman (2005), and Luby et al. (2014). The NNB contains values for vitamin E levels in fresh carrot and for some commercial food products that contain carrot. In this study, we evaluated tocochromanol levels in commercially available carrots and food products containing carrot to obtain a fuller picture of vitamin E levels in this vegetable, and compared our findings to published levels for vitamin E in carrot and to those in the NNB.

## Materials and Methods

We purchased nine different commercially available food products containing carrots, including Just Veggies organic dehydrated carrots, Freshlike frozen sliced carrots, Clear Value whole canned sliced carrots, S&W canned julienne carrots, Whole Foods organic baby carrots, Copps fresh whole carrots, Whole Foods organic fresh whole carrots, Earth's Best organic baby food with carrots, and Fresh Market organic carrot juice. All products were purchased in Madison, WI. For whole carrots, a sample of root  $\approx 2$  cm thick was removed from the top of the root, lyophilized, and ground into a powder. For commercial food products, a similar volume of material was lyophilized and ground. All samples were extracted and analyzed in duplicate. Samples were stored at  $-80^\circ\text{C}$  until HPLC analysis.

A modified protocol from Bligh and Dyer (1959) was used, based on the protocol of Maeda et al. (2008), where 50 mg of lyophilized carrot tissue or carrot food product was added to a 2-mL Eppendorf tube with 3 (3 mm) glass beads, 400  $\mu\text{L}$  of 2:1 methanol to chloroform (v/v) with 0.01% butylated hydroxytoluene (BHT), and tocol standard (rac-5,7 dimethyltolcol; Matreya, Pleasant Gap, PA). Samples were shaken using a Genogrinder for 3 min at a setting of 500 strokes/min. An additional 300  $\mu\text{L}$   $\text{H}_2\text{O}$  and 125  $\mu\text{L}$  chloroform were then added to the homogenates, which were further shaken for 30 s at the same settings. After phase separation by

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Table 1. Mean values, in  $\mu\text{g}\cdot\text{g}^{-1}$  fresh weight, and standard deviations (sd) of  $\alpha$ -tocopherol (AT),  $\gamma$ -tocopherol (GT),  $\alpha$ -tocotrienol (ATCT),  $\gamma$ -tocotrienol (GTCT), and total tocopherols and tocotrienols (TOT) measured in  $\mu\text{g}\cdot\text{g}^{-1}$  fresh weight via high-performance liquid chromatography in nine commercial food products.

Product	AT	SD	GT	SD	ATCT	SD	GTCT	SD	TOT	SD
Just Veggies organic dehydrated carrot	0.00560	0.00020	0	0	0.00162	0.00026	0.00027	0.00039	0.00749	0.000004
Freshlike frozen, sliced carrots	0.03024	0.00034	0.00105	0.000060	0.00565	0.00030	0.00029	0.00040	0.03722	0.0000002
Clear Value canned, sliced carrots	0.05077	0.00232	0.00171	0.000007	0.01185	0.00032	0.00072	0	0.06505	0.0000026
S&W canned julienne carrots	0.02562	0.00133	0.00122	0.000020	0.00932	0.00005	0.00063	0.00002	0.03679	0.0000017
Whole Foods baby organic carrots	0.01368	0.00050	0.00047	0.000020	0.00395	0.00019	0	0	0.01801	0.0000004
Copps whole carrots	0.01823	0.00500	0.00104	0.000054	0.00193	0.00002	0.00249	0	0.02369	0.0000065
Whole Foods organic whole carrots	0.02080	0.00112	0.00109	0.000016	0.00840	0.00008	0.00050	0.00003	0.03079	0.0000015
Earth's Best organic carrot baby food	0.02264	0.00319	0.00099	0.000031	0.00792	0.00001	0.00071	0.00008	0.0323	0.0000043
Fresh Market organic carrot juice	0.01507	0.00319	0.00097	0.000039	0.00544	0.00010	0.00060	0.00008	0.02208	0.0000040

centrifugation for 2 min at 21,100 g, 195  $\mu\text{L}$  of the organic phase was transferred to a new Eppendorf tube and dried down using a centrifuge concentrator (Labconco, KS City, MO) for 25 min at 30  $^{\circ}\text{C}$  (lowest temperature setting). The dried samples were then resuspended in 150  $\mu\text{L}$  of MeOH with 0.05% BHT. Five microliters of each sample was injected into the HPLC (Agilent 1260; Agilent, Santa Clara, CA) equipped with a Zorbax eclipse XDB-C18 reverse phase column ( $3 \times 150$  mm, 5  $\mu\text{m}$ ) using a 20 min isocratic elution of 95% methanol and 5% water at a flow rate of 1.0  $\text{mL}\cdot\text{min}^{-1}$  (Luby et al., 2014). Tocochromanols were detected by fluorescent detection (290 nm excitation, 330 nm emission) and quantified against standard curves generated from commercially available  $\alpha$ -,  $\gamma$ -tocopherol and  $\alpha$ - and  $\gamma$ -tocotrienol standards (Sigma Aldrich, St. Louis, MO; Cayman Chemical, Ann Arbor, MI). Commercially available standards for other tocopherol isomers were cost prohibitive. The four tocochromanols measured were summed to get a total tocochromanol level for each carrot food sample.

## Results and Discussion

When converted to a fresh weight basis, carrot and carrot food products ranged from 0.007 to 0.065  $\mu\text{g}\cdot\text{g}^{-1}$  total tocochromanols (Table 1), a finding that was generally lower but close to other recently published levels for tocochromanols in carrot (Burns et al., 2003; Koch and Goldman, 2005; Luby et al., 2014). Clear Value sliced carrots were highest in total tocochromanols, while Just Veggies dehydrated organic carrots were lowest. Canned, frozen, and fresh whole carrots had similar levels of total tocochromanols, but all nine products had levels that were lower than hybrid carrots reported by Luby et al. (2014), and none exceeded 0.065  $\mu\text{g}\cdot\text{g}^{-1}$  total tocochromanols.

Other studies have reported tocochromanol concentrations in carrot to be significantly higher than the results reported here and by Koch and Goldman (2005), Burns et al. (2003), and Luby et al. (2014). Ombodi et al. (2014) found values of 21.7–36.6  $\text{mg}\cdot\text{kg}^{-1}$  total tocochromanols on a dry weight basis using HPLC for carrots grown at different levels of irrigation. The fresh weight equivalent for these measurements for total tocochromanols ranged from 3.31 to 4.72  $\text{mg}\cdot\text{kg}^{-1}$ . Metzger and Barnes (2009) found

levels from 95 to 110  $\mu\text{g}\cdot\text{g}^{-1}$   $\alpha$ -tocopherol on a dry weight basis in a range of carrot cultivars that were being assessed for various polyacetylene compounds. Their measurements were based on gas chromatography-mass spectrometry (GC-MS), a method that has not been used for measurement of tocochromanols from carrot other than in their study. Nicolle et al. (2004) found levels of 1.91–7.03  $\text{mg}\cdot\text{kg}^{-1}$  total tocopherols on a fresh weight basis, which were relatively similar to levels reported by Ombodi et al. (2014).

The NNB is produced by the USDA (2014). This searchable and downloadable database serves as a standard for the nutrient composition of foods and is used widely to make dietary recommendations. Vitamin E values on a fresh weight basis for raw carrot are reported in the NNB as 6.6  $\mu\text{g}\cdot\text{g}^{-1}$  and vitamin E levels in other food products containing carrot are reported at similar levels, such as 5.6  $\mu\text{g}\cdot\text{g}^{-1}$  for carrot juice. If the NNB data are taken at face value, these vitamin E values for raw carrot are significantly higher than experimentally determined values published in three independent studies (Burns et al., 2003; Koch and Goldman, 2005; Luby et al., 2014) as well as in the data we report here. Those NNB data for vitamin E in carrot are also higher than levels reported by Ombodi et al. (2014), although by a far lesser amount. This discrepancy suggests that vitamin E levels in carrot are not clear. If the lower values are correct, carrots do not contain enough vitamin E to be nutritionally significant. If the higher values are correct, nutritional recommendations for vitamin E in carrot may need to be clarified in various publications to reflect their true nutritional value.

The recommended daily intake (RDI) of vitamin E in adults is 15 mg (Anonymous, 2014) whereas in one study, the vitamin E levels in carrot averaged 17  $\mu\text{g}$  in a 128 g serving of carrot (Luby et al., 2014, reported as an average of hybrid carrot varieties measured, adjusted to 80% moisture level), and lower, depending on the carrot food product. If these lower levels are correct, carrot would not appear to be a significant source of dietary vitamin E as a serving only provides  $\approx 0.1\%$  of the RDI. The amount of  $\alpha$ -tocopherol on a fresh weight basis in carrot root reported by Luby et al. (2014) (0.12  $\mu\text{g}\cdot\text{g}^{-1}$ ) is comparable to levels found in other vegetable crops such as lettuce (0.1  $\mu\text{g}\cdot\text{g}^{-1}$ ),

green pepper (0.1  $\mu\text{g}\cdot\text{g}^{-1}$ ), and red and yellow pepper (both 0.6  $\mu\text{g}\cdot\text{g}^{-1}$ ; Burns et al., 2003).

Food samples analyzed in this study were purchased from a single location and analyzed. This observational study was not designed to provide comprehensive data on tocochromanol levels for carrot. For a comprehensive study, food products should be sourced from appropriate geographic locations and sampled appropriate to brand market share (Perry et al., 2003). The purpose of this study was to point out the discrepancy in published values from different studies for vitamin E in carrot. This discrepancy may be due to methodological differences, cultivar differences, environmental effects, the number of tocopherols or tocotrienols measured, or some combination of these factors. Nicolle et al. (2004) used ultraviolet detection, which is not specific and may overestimate tocopherols if compounds are present that co-migrate with other tocochromanols. However, Ombodi et al. (2014) used fluorescence detection, which is the method we reported here, but their levels were similar to those reported by Nicolle et al. (2004). Thus, similar methods produced quite different results in separate studies. Saponification was used by Ombodi et al. (2014), but not Nicolle et al. (2004), and they reported similar values. It is also possible that because eight compounds comprise the tocochromanol portfolio, the measurement of different suites of these compounds in each study further compounds the lack of correspondence among the published works. Further study should be undertaken to clarify this discrepancy, which should promote greater accuracy in nutritional recommendations.

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