Journal of Food Composition and Analysis 23 (2010) 253–259

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Article

# Stability of vitamin C in frozen raw fruit and vegetable homogenates

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ARTICLE INFO

*Article history:*
Received 20 March 2009
Received in revised form 15 August 2009
Accepted 26 August 2009

*Keywords:*
Vitamin C
Ascorbic acid
Dehydroascorbic acid
Freezing
Temperature
Stability
Storage
Homogenization
Analysis
Fruits
Vegetables
Oranges
Juice
Clementines
Tangerines
Citrus
Collards
Potatoes
Leafy greens
Food analysis
Food composition

ABSTRACT

Retention of vitamin C in homogenized raw fruits and vegetables stored under routine conditions prior to analysis was investigated. Raw collard greens (*Brassica oleracea* var. viridis), clementines (*Citrus clementina* hort. ex Tanaka), and potatoes (*Solanum tuberosum*) were chosen, being representative of foods to be sampled in USDA's National Food and Nutrient Analysis Program (NFNAP), and having different expected stability of ascorbic acid (AA). Samples were homogenized in liquid nitrogen, assayed immediately, then stored at −60 °C and analyzed at time points up to 49 weeks. Vitamin C (as total AA after reduction of dehydroascorbic acid) was analyzed using a validated method with quantitation by HPLC/ultraviolet detection. An orange juice control sample was included in each run. Vitamin C concentrations were stable in clementines and the orange juice, but decreased in collards and potatoes [16.8 and 10.9 mg/100 g (14.7% and 30.4%), respectively, after 49 weeks]. Significant losses had occurred after 12 weeks. These results suggest similar matrices must receive careful attention to sample handling protocols before analysis or AA values may not reflect the concentration in the food as consumed. The control sample was critical to allowing assessment of storage effects independent of analytical variability. Fruits and vegetables for the NFNAP will be analyzed without storage until a practical stabilization protocol is validated.



## 1. Introduction

The nutritional importance of vitamin C (L-ascorbic acid; 2,3-endiol-L-gulonic acid-γ-lactone) as an essential water-soluble vitamin is well established. It has long been known that a nutritional deficiency in vitamin C causes scurvy, a disease characterized by bleeding gums, impaired wound healing, anemia, fatigue, and depression, that, without proper care, can eventually be fatal (Davies et al., 1991; Arrigoni and De Tullio, 2000). Ascorbic acid (AA) is a cofactor in numerous physiological reactions, including the post-translational hydroxylation of proline and lysine in collagen and other connective tissue proteins, collagen gene expression, synthesis of norepinephrine and adrenal hormones, activation of many peptide hormones, and synthesis of carnitine (Bender, 2003; Johnston et al., 2007). Also, due to its redox potential, ascorbic acid facilitates intestinal absorption of iron and functions as a cellular antioxidant alone and coupled to the antioxidant activity of vitamin E (Byers and Perry, 1992; Bender, 2003). Therefore, adequate intake of vitamin C from foods and/or supplements is vital for normal functioning of the human body.

Recommended Dietary Allowances (RDA) of 75 mg/day and 90 mg/day have been established for adult women and men, respectively, and 45 mg/day for children 9–12 years old (Food and Nutrition Board, Institute of Medicine, 2000). Recent interest in the role of dietary antioxidants in general, and of specific food components, requires accurate food composition data to facilitate epidemiological studies and feeding trials relating the intake of vitamin C to physiological effects, and to develop food consumption recommendations.

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doi:10.1016/j.jfca.2009.08.018

Vegetables and fruits, particularly citrus fruits, green leafy vegetables, broccoli, cauliflower, Brussels sprouts, tomatoes, peppers, and potatoes, are major food sources of vitamin C (Eitenmiller et al., 2008). However, vitamin C is subject to oxidative and enzymatic degradation to dehydroascorbic acid (DHAA) and also irreversible oxidation via DHAA to diketogulonic acid, and the latter has no vitamin C activity (Nyyssonen et al., 2000). Ascorbic oxidase is the endogenous enzyme involved in this process (Saari et al., 1995). Various factors, including the presence of oxygen and metal ions (especially $Cu^{2+}$, $Ag^{+}$, $Fe^{3+}$), alkaline pH, and high temperature affect the vitamin C content of raw produce prior to the point of consumption and result in variation in the actual levels in different samples of a given product (Lee and Kader, 2000). Light, pH, temperature, oxygen exposure, the presence of oxidizing metals, and oxidizing enzymes can be controlled during the assay itself, but must also be controlled during preparation of samples for analysis, especially if the procedures involve maceration or other disruption of cells which release oxidizing enzymes. Failure to assess stability of vitamin C in raw produce during sample processing and analysis could result in significant errors in analytical results.

The primary source of food composition data in the United States is the U.S. Department of Agriculture's (USDA) National Nutrient Database for Standard Reference (SR) (USDA, 2008). The USDA National Food and Nutrient Analysis Program (NFNAP) is an ongoing project to update and improve the quality of food composition data in SR (Haytowitz et al., 2008). For the aforementioned reasons, vitamin C in many fruits and vegetables was identified as a key nutrient requiring attention. One of the practical challenges in the NFNAP is that a wide range of nutrients must be assayed in each sample procured, and, furthermore, numerous primary samples must be obtained to represent the national supply of a given food (Pehrsson et al., 2000). The cost of purchasing, shipping, and preparing samples for analysis is a significant factor in the total cost of the project. There is a fundamental need to standardize and document the handling of samples via a complete audit trail from sample procurement to the release of final data in SR, and archived subsamples of all composites must be maintained as well. Therefore, centralized sample preparation is a practical approach for the NFNAP. Primary food samples (sample units) are procured from retail and wholesale locations and are sent to a laboratory [the Food Analysis Laboratory Control Center (FALCC) at Virginia Tech, Blacksburg, VA] where they are prepared, composited, homogenized, and dispensed into subsamples that are distributed for analysis along with quality control materials (Phillips et al., 2006). Because analytical values are used to estimate nutrient values in the product at point of consumption, it must be ensured that degradation of nutrients does not occur during the preparation process, e.g., homogenization, subsampling, and storage of samples prior to analysis. The degree of nutrient loss during standard storage conditions must be verified for labile nutrients. Under routine NFNAP processing conditions, a minimum of 2 weeks, and often several weeks, elapse between homogenization and analysis. Additionally, it was necessary to determine if vitamin C content of archive samples stored for longer periods would still be representative of the original sample.

Previously the stability of folate in raw fruit and vegetable homogenates prepared for NFNAP analysis was established (Phillips et al., 2005). In an initial study of vitamin C in raw produce, results for some products were unexpectedly variable and/or lower than expected (Fig. 1) for some raw fruits, with some values much less than half of the vitamin C concentrations reported in Release 14 of SR (USDA, 2001). Those values were not used to update SR, and reasons for the discrepancies were considered, including stability during sample storage. While it is known that degradation of vitamin C can occur in homogenates of raw produce, literature on the stability of vitamin C in fruits and vegetables cannot be directly or definitively extended to the NFNAP foods and sample storage protocol. For example, Gonzalez et al. (2003) measured vitamin C in raspberries and blackberries stored from 0 to 12 months and found an average decrease of 37% and 31% (10.7 and 7.9 mg/100 g), respectively, but the storage temperature of −24 °C was higher than the −60 °C used under NFNAP protocols, and the berries were frozen whole, not homogenized. Vanderslice et al. (1990) reported on the vitamin C content of selected fruits and vegetables and performed stability testing on raw broccoli samples stored under different conditions (refrigerated at −4 °C and frozen at −40 °C, with or without citric acid or metaphosphoric acid). The treatment in the Vanderslice et al. (1990) study that is most relevant to NFNAP standard conditions (−60 °C under nitrogen) was storage at −40 °C. In that

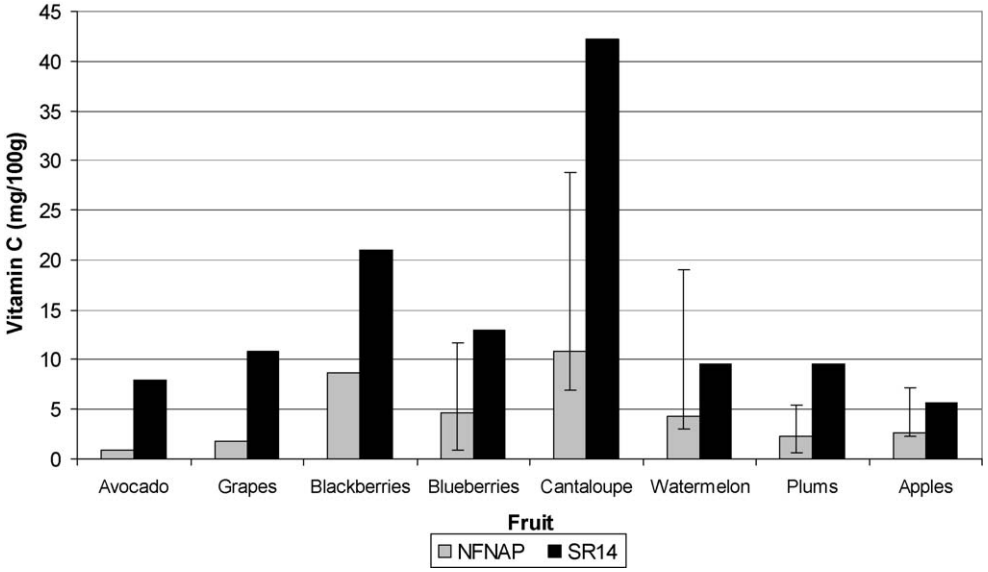


**Fig. 1.** Preliminary analytical results for vitamin C in selected fresh fruits sampled for NFNAP in 2001–2002, compared to Release 14 of the USDA Nutrient Database for Standard Reference (SR14) (USDA, 2001). Values plotted are the average for 4 samples, and error bars represent the range.