



## Original Article

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

Katherine M. Phillips<sup>a,\*</sup>, Maria Teresa Tarragó-Trani<sup>a</sup>, Susan E. Gebhardt<sup>b</sup>, Jacob Exler<sup>b</sup>,  
Kristine Y. Patterson<sup>b</sup>, David B. Haytowitz<sup>b</sup>, Pamela R. Pehrsson<sup>b</sup>, Joanne M. Holden<sup>b</sup>

<sup>a</sup> Department of Biochemistry (0308), 304 Engel Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0308, USA

<sup>b</sup> USDA ARS, Beltsville Human Nutrition Research Center, Nutrient Data Laboratory, 10300 Baltimore Blvd., Bldg. 005, Room 107, Beltsville, MD 20705, USA

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## 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  $-60\text{ }^{\circ}\text{C}$  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.

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## 1. Introduction

The nutritional importance of vitamin C (L-ascorbic acid; 2,3-endiol-L-gulonic acid- $\gamma$ -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 norepi-

nephrine 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.

\* Corresponding author. Tel.: +1 540 231 9960; fax: +1 540 231 9070.

E-mail addresses: [kmpvpi@vt.edu](mailto:kmpvpi@vt.edu), [kp.jfca@yahoo.com](mailto:kp.jfca@yahoo.com) (K.M. Phillips).

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  $\text{Cu}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Fe}^{3+}$ ), 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  $-24\text{ }^{\circ}\text{C}$  was higher than the  $-60\text{ }^{\circ}\text{C}$  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  $-4\text{ }^{\circ}\text{C}$  and frozen at  $-40\text{ }^{\circ}\text{C}$ , 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 ( $-60\text{ }^{\circ}\text{C}$  under nitrogen) was storage at  $-40\text{ }^{\circ}\text{C}$ . In that

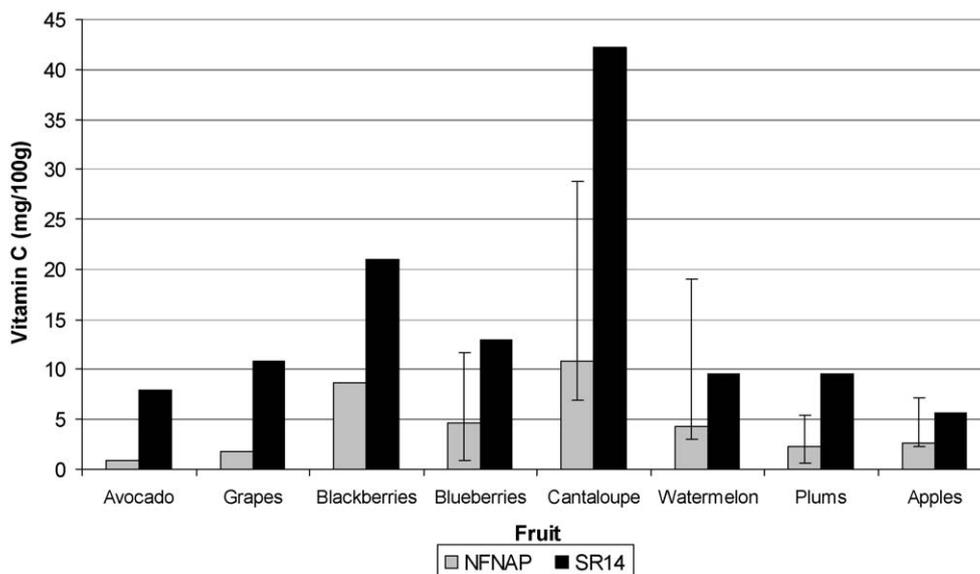


Fig. 1. Preliminary analytical results for vitamin C in selected fresh fruits sampled for NFNAP in 2001–2002, compared to Release 14 of the USDA Nutrient Database for Standard Reference (SR14) (USDA, 2001). Values plotted are the average for 4 samples, and error bars represent the range.

study, total vitamin C as ascorbic acid plus DHAA was constant for 2 weeks ( $133 \pm 7$  mg/100 g) and dropped thereafter, reaching  $89 \pm 25$  mg/100 g after 2 months (Vanderslice et al., 1990). However, the lower temperature and nitrogen atmosphere used in NFNAP should impart additional stability. Furthermore, because pH and other matrix-specific characteristics are known to affect vitamin C stability (Musulin and King, 1936; Moser and Bendich, 1990; Wechtersbach and Cigic, 2007), results for broccoli might not apply equally to other of fruits and vegetables.

While homogenization in citric acid or metaphosphoric acid, as reported for broccoli by Vanderslice et al. (1990), might stabilize vitamin C, this special treatment would be impractical in NFNAP because it would require a separate composite just for this nutrient. The number of samples and composites to be prepared would be effectively doubled. Furthermore, the number of samples to be analyzed exceeds the total that can be assayed in a single batch by the average laboratory, delaying the analysis of some samples.

The objective of this study was to evaluate the retention of vitamin C in frozen homogenates of representative raw fruits and vegetables held over a period of time under the typical processing and storage methods for NFNAP.

## 2. Materials and methods

### 2.1. Overview of the study

The experimental design accommodated the number of analyses that could be performed in one assay batch of practical size (10 samples, including replicates and control samples) per time point, and therefore comprised three foods. Collard greens (*Brassica oleracea* var. *viridis*), clementines (*Citrus clementina* hort. ex Tanaka) (a seasonal substitute for tangerines), and russet potatoes (*Solanum tuberosum*) were chosen after reviewing NFNAP Key Foods list (Haytowitz et al., 2002), data for representative types of fruits and vegetables and considering different matrix characteristics [including pH (estimated by published values, U.S. Food and Drug Administration, 2007), starch content, plant part, etc.] that might affect stability of ascorbic acid. Although the selected vegetables are usually eaten cooked, the purpose of this study was to assess matrices with representative characteristics that might affect vitamin C stability in raw produce, and reasonably assume that the results could be extended to similar fruits and vegetables. Also, it was assumed that effects of enzyme activity would be greater in uncooked plant materials; therefore, raw samples were used.

Composites were prepared following the standard NFNAP protocols for fruits and vegetables [homogenization in liquid nitrogen using a Robot Coupe Blixer food processor (Robot Coupe USA, Jackson, MS) and storage of subsamples at  $-60 \pm 5$  °C under nitrogen in sealed glass jars] that was previously shown to yield homogeneous subsamples (Phillips et al., 2006) and maintain moisture content (Phillips et al., 2001). Vitamin C was assayed immediately after homogenization, and then in subsamples that were stored at  $-60 \pm 5$  °C. Each composite was assayed in triplicate at each of 7 additional time points (0.5, 1, 2, 3, 4, 6 and 12 months) over a period of 1 year. Precise kinetic studies were not conducted because the goal was to assess stability after storage periods that afforded some practical advantage over analysis immediately after homogenization.

### 2.2. Samples, composite preparation and subsample storage

Clementines (~2.3 kg), raw collards (~1.3 kg), and russet potatoes (~2.0 kg) were procured locally (Blacksburg, VA). The

sample units for each composite were prepared immediately prior to homogenization. All work was performed under ultraviolet-filtered light. The collards were rinsed with distilled deionized (DDI) water for 2 min, drained in a plastic colander, and patted dry with lint-free towels. The leafy green portions were separated from the tough fibrous stalks and the latter were discarded; any parts of the leaves that appeared dark brown and inedible were also removed. The greens were cut into ~1.25 cm squares using a stainless steel knife, then immediately frozen in liquid nitrogen. Potatoes were rinsed with DDI water for 2 min, drained in a plastic colander and patted dry with lint-free towels. No refuse was removed; the unpeeled potatoes were chopped into ~1.25 cm cubes with a stainless steel knife, then frozen immediately in liquid nitrogen. For clementines, 10 fruits were randomly selected from each bag or box and each was peeled and sectioned. Refuse (peel and any significant albedo that adhered to the fruit) was removed, and the fruit sections were frozen immediately with liquid nitrogen.

For each food composite the material frozen in liquid nitrogen was transferred to a 6L stainless steel food processor (Robot Coupe 6L Blixer; Robot Coupe USA, Jackson, MS), blended 10 s at 1500 rpm, then for one to four additional 30 s periods at 3500 rpm, until a homogeneous composite resulted. Additional liquid nitrogen was added as necessary to keep the composite frozen. For each composite the ground material was transferred to a stainless steel bowl and kept frozen by adding and stirring in additional liquid nitrogen as needed, and dispensed among twenty-four 60-mL glass jars with Teflon™-lined lids (~10 g/jar, filled half full). Jars were wrapped with aluminum foil and stored at  $-60$  °C in darkness.

### 2.3. Analysis of vitamin C

Vitamin C was analyzed as AA by HPLC with UV detection, after reduction of DHAA to AA.

### 2.4. Reagents and standards

ACS grade ascorbic acid ( $\geq 99\%$ ), metaphosphoric acid (MPA), ethylenediaminetetraacetate disodium salt (EDTA), and formic acid were purchased from Sigma–Aldrich (Saint Louis, MO). Tris(2-carboxyethyl) phosphine (TCEP) was obtained from Thermo Scientific (Rockford, IL). HPLC grade methanol and water were purchased from Fisher Scientific (Pittsburgh, PA).

### 2.5. Extraction

All sample preparation was performed in a room in which UV light was filtered. Vitamin C was quantified as total AA after treatment of samples with TCEP to reduce any DHAA to AA, by inclusion of TCEP in the extraction buffer. The concentration of TCEP represented a 5- to 20-fold molar excess for samples with the highest AA concentration; a 5-fold excess has been shown to yield  $99 \pm 1\%$  conversion of DHAA to AA (Wechtersbach and Cigic, 2007).

A representative portion of the ground material ( $2 \pm 0.1$  g) was weighed into a 50 mL Teflon™ centrifuge tube (Fisher Scientific, Pittsburgh, PA, cat. # 05-529C), and 10 mL of extraction buffer [5% MPA/1 mM EDTA/5 mM TCEP, pH 1.55] was added. For the initial analyses, the subsample of the homogenized material, still frozen in liquid nitrogen, was weighed into the extraction tube immediately after homogenization, then 8 mL of extraction buffer was added and the tube was set on ice and extracted within 1 h. For intermediate time points, composite subsamples were removed from the  $-60$  °C freezer immediately before analysis and then kept on ice during mixing and subsampling. The samples in buffer were homogenized

using an Omni™ mixer fitted with a saw tooth generator blade, 10 mm × 195 mm (Omni International Cat. # 17105 and # 15010, Marietta, GA) for 2 min. Tubes were kept on ice before and after homogenization. During homogenization the centrifuge tube was submerged in ice water, with a flow of argon on top of the homogenate. Samples were centrifuged at 10 °C and 5000 rpm (7280 × g) for 30 min. The supernatant was decanted into the top chamber of a 25 mL Maxi-Spin™ centrifuge filter tube with 0.45 μm polyvinylidene fluoride (PVDF) membrane (Alltech/Grace, cat. # 24162, Deerfield, IL), capped under argon and kept on ice. The pellet was thoroughly resuspended in 5 mL of extraction buffer, capped under argon, sonicated for 5 min, then centrifuged for 30 min at 10 °C and 5,000 rpm. The second supernatant was combined with first and then filtered through the 25 mL Maxi-Spin™ centrifuge filter tube with 0.45 μm PVDF membrane. The filtered extract was quantitatively transferred to a 25 mL volumetric flask, taken to volume with extraction buffer, capped under argon, and thoroughly mixed. The solution was then transferred to a 40 mL amber glass vial with Teflon™ coated cap, capped under argon, and stored at –60 °C until analysis (within 1 week).

Extracts were thawed by first placing the vials in ice water for about 30 min, then replacing the ice water with room temperature water, allowing samples to equilibrate for an additional 30 min, and then the solutions were diluted with extraction buffer in the range of 1/5 to 1/20, depending on the estimated AA concentration in the samples (orange juice and clementine extracts were diluted 1/10, and collard greens and potatoes 1/20). Subsequently 1 mL aliquots were transferred to 2 mL amber HPLC/GC vials, capped under argon and stored at –60 ± 5 °C until analyzed by high performance liquid chromatography (HPLC) within 24 h.

## 2.6. HPLC analysis

HPLC was performed using a C18 reversed-phase column with polar end-capping [Synergi™ 4m Hydro-RP (250 mm × 4.6 mm, 4 μm particle size); Phenomenex, Torrance, CA] and a mobile phase of 0.05% aqueous formic acid. The HPLC system consisted of a binary pump 250 and a diode array detector (model 235C) operated with Turbochrom™ 4 program (PerkinElmer, Waltham, Massachusetts). Immediately prior to HPLC analysis each sample was filtered through a Mini-Uniprep® syringeless filter with 0.45 μm PVDF-L membrane (Whatman® cat. #UN203APUAQU, Florham Park). Twenty μL of filtered, diluted extract (filtered previously through a 0.45 μm nylon filter) was injected into the HPLC, and eluted under isocratic conditions at 1 mL/min. AA was detected with a photodiode array detector at 255 nm. Under these conditions the AA peak eluted around 5.2–5.5 min. AA standards in the range of 0.5–50 μg/mL were run on the HPLC with every assay as external standards, and the calibration curve was used to quantify AA in the samples. The limit of quantification (LOQ) was approximately 0.15 μg/mL which corresponded to ~0.19 mg/100 g AA in samples.

## 2.7. Quality control

The method was specifically evaluated to verify lack of interference in the matrices studied. Testing included making sure there were no obvious interferences in the ascorbic acid peak. Trial composites were prepared from locally procured samples of the same type planned for the stability study and extracted and analyzed as described above. Using the program Turboscan™ (PerkinElmer) for the photodiode array detector, the peak purity based on comparison of the UV absorption spectrum of the AA peak at 3 different points of elution (upslope, downslope, and baseline) to the peak of the authentic AA standard. If the peak was not well resolved from interferences in a particular matrix, dilutions were

assessed to determine if interfering peaks could be reduced without compromising the LOQ needed.

A control sample (pasteurized commercial orange juice), with 40.45 ± 2.01 mg/100 g as the tolerance limits for vitamin C (established as described by Taylor, 1987), was included in each analytical batch. This material had been dispensed in 40–45 mL portions in 60 mL polypropylene bottles and stored at –60 °C for use as an in-house control material and was previously established to have a stable vitamin C concentration under these conditions (unpublished data, M.T. Tarragó-Trani, 2007).

## 2.8. Data analysis

Data analysis, statistics, and plots were executed using Microsoft® Excel for Apple® Macintosh version 11.1.0®, 2004 (Microsoft® Corporation, Redmond, WA) except where mentioned otherwise.

Control sample data were used to account for natural analytical variability across runs, and to separate it from stability in vitamin C concentration in samples over time (Phillips et al., 2005). The vitamin C concentration measured after homogenization, with no thawing allowed, was considered the initial concentration. Stability was assessed by comparing the concentration at the final time point to the initial concentration using a Student's *t*-test (Quattro Pro®, version 14.0.0.603; Corel Corporation, Ottawa, Ontario, Canada). The trend across intermediate time points was estimated using a best-fit polynomial regression using Excel®, with the assumption that AA would not decrease then increase across subsequent time points. Thus any increases in the mean value at intermediate time points, in the context of an overall decrease over time, was considered to be analytical uncertainty and not a change in AA concentration in the samples.

## 3. Results and discussion

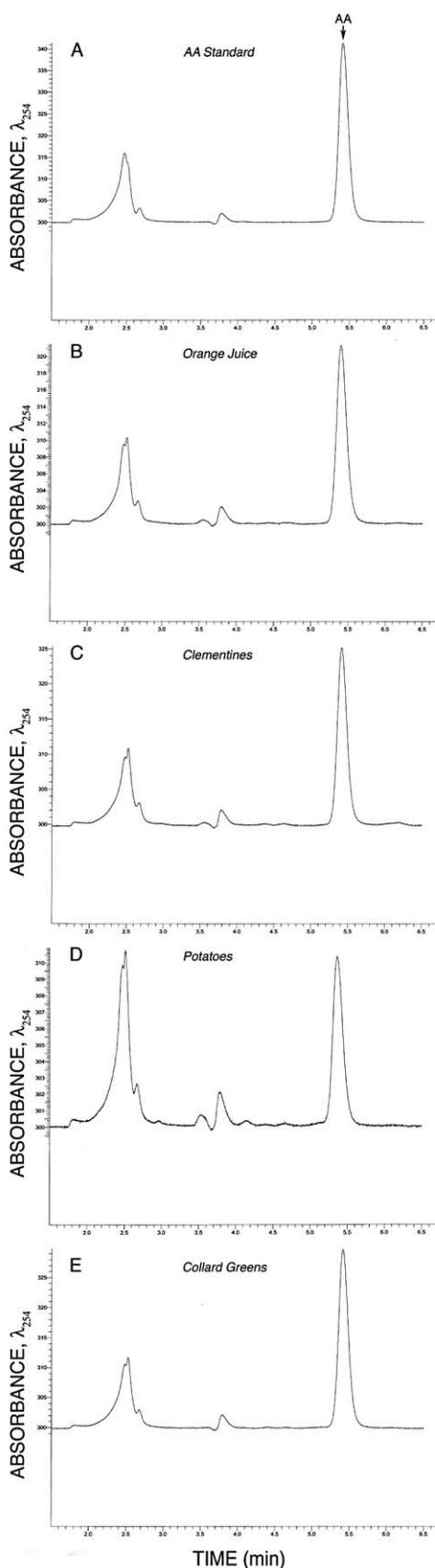
### 3.1. Method validation for the matrices studied

The clementine chromatograms showed a well-resolved AA peak, with no difference in peak purity relative to the AA standard. On the other hand, initially the collard greens and potatoes chromatograms showed a clearly defined AA peak but with some minor (unidentified) shoulder peaks that were not completely resolved from the AA peak. This interference was eliminated effectively with a 1/20 concentration instead of the initial 1/5 dilution for both the collard greens and potatoes, with the diluted samples showing a clean AA peak, and the peak purity checked with Turboscan™ was comparable to that of AA standard. Representative chromatograms are shown in Fig. 2.

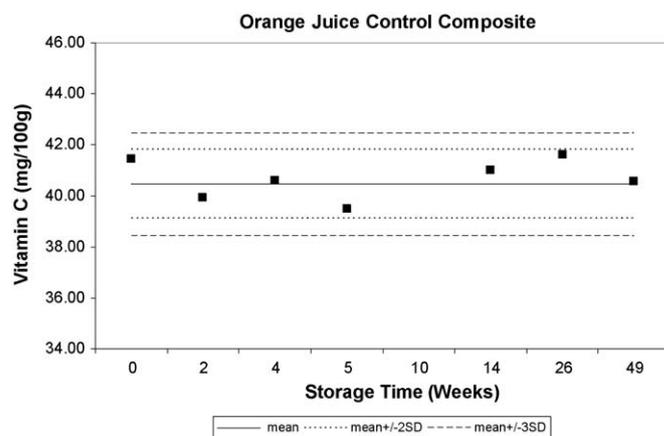
### 3.2. Quality control

Fig. 3 shows the control chart for the orange juice control sample that was established prior to the study, along with the data obtained for this material when assayed with these samples throughout the storage period. The mean and relative standard deviation (RSD) for the preliminary assays used to set the limits shown in Fig. 3 (*n* = 20 over 15 separate assay batches) were 40.4 mg/100 g and 1.7%. The mean and RSD for the 7 study data points were 40.65 mg/100 g and 1.9%, with all values falling well within the tolerance limits and centered around the mean.

The inclusion of a control sample in each analytical batch was critical to establish that any changes were due to stability of vitamin C in a particular sample and not natural day-to-day analytical variability or gross error in a particular sample batch.



**Fig. 2.** Representative chromatograms for ascorbic acid standard (A), orange juice (B), clementines (C), potatoes (D), and collard greens (E). Chromatography conditions: C18 reversed-phase column with polar end-capping (250 mm × 4.6 mm, 4 μm particle size); mobile phase of 0.05% aqueous formic acid, isocratic at 1 mL/min; 20 μL injection volume; detection at 255 nm with a photodiode array detector.



**Fig. 3.** Results for the control material (orange juice) assayed with each set of samples throughout the stability study. The mean and tolerance limits are based on assays prior to the stability study ( $n = 20$  over 15 separate assay batches). SD = standard deviation. "Storage time" refers to the storage time for samples, shown in Fig. 4.

Control samples should always be included when conducting seasonal or stability studies, and the analytical uncertainty must be included in any evaluation of changes attributed to sample characteristics or treatment variables.

### 3.3. Stability of vitamin C in homogenates stored at $-60^{\circ}\text{C}$

Fig. 4 illustrates the vitamin C content of the stored homogenized samples over time at  $-60^{\circ}\text{C}$ . Table 1 summarizes the decrease in AA concentration at various storage times for the processed collard greens and potatoes, as estimated from the regression equations (Fig. 4). There was no change in the vitamin C concentration in clementines after 1-year storage ( $p = 0.66$ ,  $\alpha = 0.05$ ). These results support a reasonable conclusion that vitamin C is stable in homogenates of raw citrus fruits stored under the conditions used in this study, probably due to the high acidity of this citrus fruit. On the other hand, there was a notable decrease ( $p < 0.0001$ ) in the assayed vitamin C concentrations in collard greens ( $-15.4$  mg/100 g) and potatoes ( $-8.8$  mg/100 g) ( $-13.7\%$  and  $-26.0\%$ , respectively) after 1 year. The results obtained immediately after homogenization were therefore considered to accurately represent the original food samples. Loss of vitamin C was minimal up to 4 weeks [1–2 mg/100 g (1–2%)] but the vitamin loss continued to accumulate thereafter. Thus storage of the homogenized samples at  $-60^{\circ}\text{C}$  (in darkness under nitrogen) for 4 weeks or less is probably acceptable for many applications, especially when balanced against the practical cost of analysis immediately after homogenization or other logistical concerns.

On a mg/100 g basis the decreases were more rapid and larger overall for collard greens. In terms of the average adult RDA of 82.5 mg for vitamin C (Food and Nutrition Board, Institute of Medicine, 2000), the underestimation of vitamin C intake from typical servings of potatoes and collard greens would be 0.6% and 2.4%, respectively, if the concentrations were determined using samples stored 4 weeks (Table 1). After 1-year storage, the deviations increased to 6.7% and 18.4% of the RDA, respectively [15.5 and 5.5 mg/serving for typical servings of 173 g for one medium-size potato and 36 g for 1 cup (250 mL) collard greens]. These are significant absolute underestimates in the actual vitamin C content at the presumed point of consumption, especially for the potatoes.

It is important to note that the present study was of stability in the products as prepared and stored prior to analysis and involved single, local samples. Therefore, the absolute vitamin C

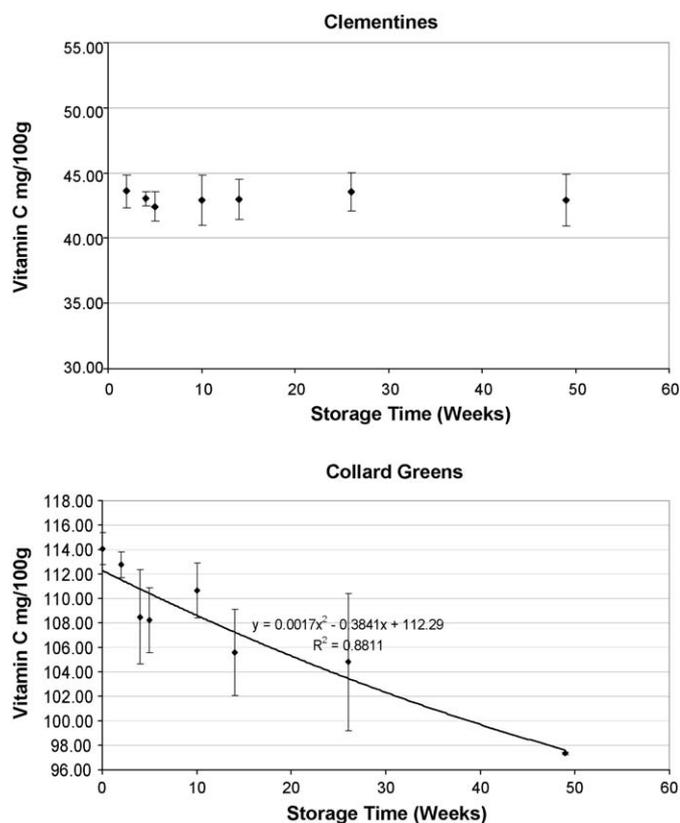


Fig. 4. Vitamin C content of homogenized raw clementines, collard greens, and potatoes during storage for 49 weeks at  $-60^{\circ}\text{C}$ . Error bars represent the 95% confidence intervals.

levels do not translate into an estimate of the vitamin C content across a representative subset of samples of these foods in the marketplace.

When literature values for vitamin C in raw fruits and vegetables are considered or new data are being generated, it is imperative to verify that sample handling prior to analysis maintains stability of the nutrient. A description of this step is missing from many published studies. The results of the present study would not necessarily extend to homogenized samples stored at higher temperatures, and temperature is a factor in stability of vitamin C, even for citrus. Burdurlu et al. (2006) showed loss of ascorbic acid in citrus juices at 28, 37 and  $45^{\circ}\text{C}$ . Additionally, if an analytical laboratory were to thaw the material prior to analysis, or possibly not homogenize samples

using liquid nitrogen, the losses could be even greater than in the current study. In a study of the vitamin C content of foods consumed in Hawaii, samples were freeze-dried prior to analysis in some cases (Franke et al., 2004). While appropriate precautions were taken by these authors, and the drying was a practical necessity due to the difficulties in sample collection and perishability, further work would be needed to fully establish that vitamin C was not reduced during sample processing compared to the food as consumed. Depending on the temperature of freezing prior to lyophilization, it is possible that vitamin C could be lost prior to drying. Food to food differences also exist. Martínez et al. (2005) found that storage of raw cut green peppers resulted in increasing vitamin C content, to levels similar to those found in ripe red peppers, but the vitamin C content of raw cut red peppers decreased. Freeze-drying did not alter the concentrations, suggesting that ripening also plays a role in the lability of vitamin C in particular products under different storage conditions.

Further complicating the picture of existing data for vitamin C is that some reports are for ascorbic acid only. AA loss may not translate to loss of vitamin C if DHAA was not also measured or reduced to AA prior to measuring vitamin C (total AA). In a study on the stability of vitamin C in refrigerated orange juice (Johnston and Bowling, 2002), while vitamin C as AA + DHAA decreased, the proportion of DHAA varied markedly among products, and represented from 51% to 91% of individual total AA concentrations.

Efforts are currently underway to update data for vitamin C in the USDA National Nutrient Database for Standard Reference, using rigorously controlled sample handling prior to analysis and carefully validated analytical methodology. Based on this study, vitamin C in NFNAP samples of raw non-citrus fruits and vegetables will be assayed immediately after compositing and homogenizing samples, to ensure maximum precision and accuracy across different samples and foods and to minimize bias. Meanwhile work will continue to develop a method for stabilization of vitamin C in homogenized raw fruits and vegetables that facilitates practical storage and handling in large scale studies such as the NFNAP. Products nationally sampled and analyzed thus far using this protocol include cauliflower, green beans, corn, green onions, yellow squash, zucchini squash, lemons, and several tropical fruits (jackfruit, cherimoya, mango, papaya, mamey sapote, feijoas), the data for which will be included in a future release of SR (starting with version 22, August 2009). Other key fruits and vegetables will be re-sampled in the upcoming year(s) for data to be included in later revisions of SR. The stored composites from this study and from additional foods, including cooked vegetables, will continue to be monitored at regular intervals.

Table 1

Decreases in vitamin C content of collard greens and potatoes after homogenization and at various storage times across a 1-year period. Concentrations are calculated from the regression equations shown in Fig. 4.

Storage time at $-60^{\circ}\text{C}$ (weeks)	Decrease in vitamin C concentration					
	Collard greens			Potatoes		
	mg/100 g	mg/serving <sup>a</sup>	Percent	mg/100 g	mg/serving <sup>b</sup>	Percent
0	0	0	0	0	0	0
2	0.8	0.3	0.7	0.4	0.7	1.3
4	1.5	0.5	1.3	0.8	1.5	2.5
8	3.0	1.1	2.6	1.7	2.9	4.9
12	4.4	1.6	3.9	2.5	4.2	7.3
16	5.7	2.1	5.1	3.2	5.6	9.5
24	8.2	3.0	7.3	4.7	8.0	13.8
52	15.4	5.5	13.7	8.8	15.2	26.0

<sup>a</sup> One medium-sized potato, 2-1/4" to 3-1/4" (5.7–8.3 cm) diameter, 173 g (USDA, 2008).

<sup>b</sup> One cup (250 mL), chopped, 36 g (USDA, 2008).

#### 4. Conclusions

In this study it was found that vitamin C in frozen homogenates of representative raw fruits and vegetables is stable for some period of time with the typical processing and storage methods used for the NFNAP. No significant change in the vitamin C content of clementines was noted over a year, but, for both potatoes and collard greens, a cumulative decrease was seen after 1 month even with storage at  $-60^{\circ}\text{C}$ , in an inert atmosphere, and protected from light. These conclusions could be made reliably based on the use of optimized methodology and analytical quality control.

In any particular study, the precision and accuracy required for analytical data should always be considered. It might be possible to accept higher uncertainty for some applications, but regardless, the potential error due to sample handling should be recognized in the overall uncertainty reported with the analytical data. Both proper quality control and optimal analytical technique are essential to minimize error and maximize precision of the data. The need for a control sample in every analytical run is extremely important, especially when conducting stability studies over time. This measure ensures that changes observed can be attributed only to samples and treatments by properly accounting for analytical uncertainty.

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